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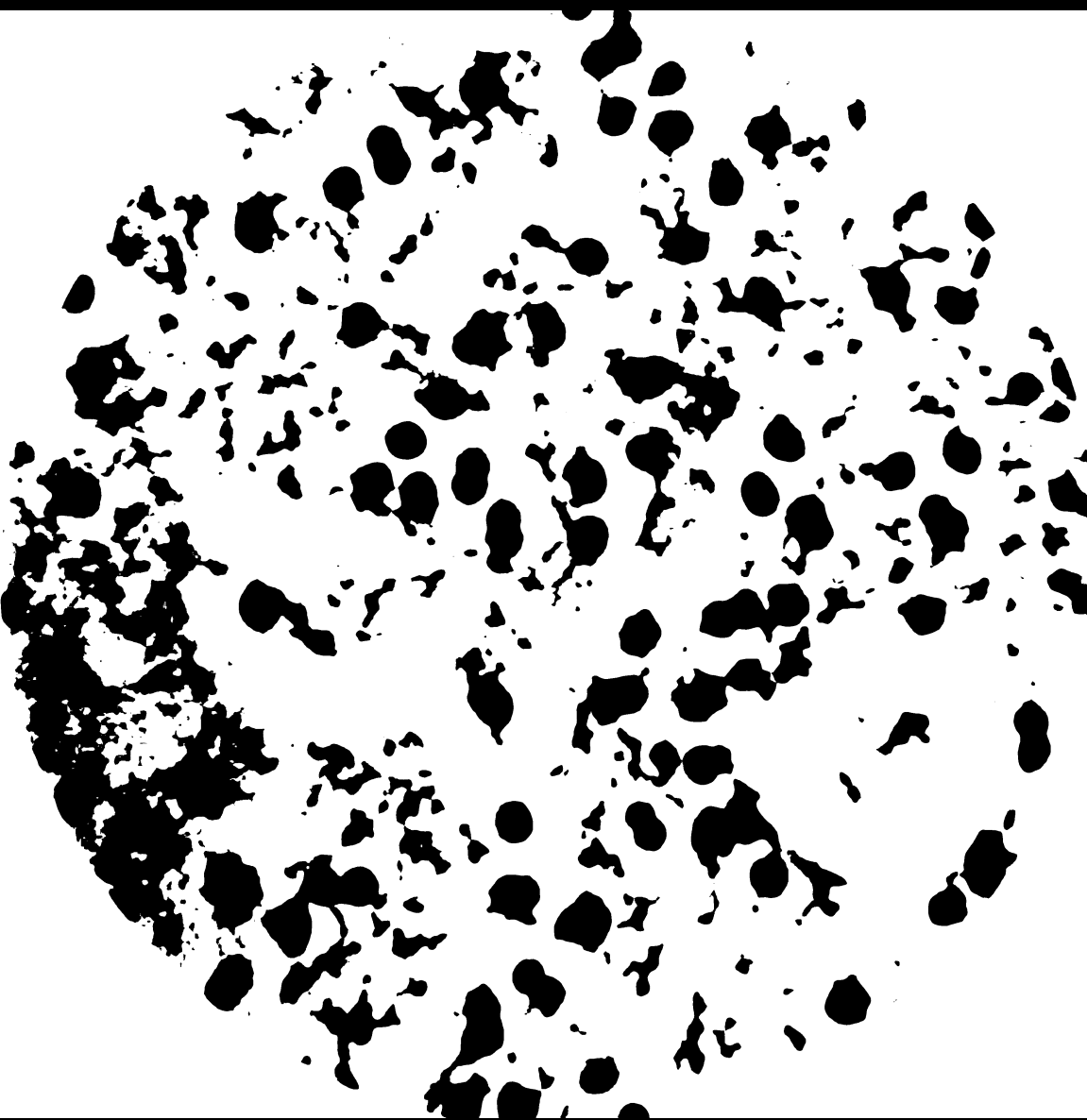
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STUDIES

FROM THE

DEPARTMENT OF PATHOLOGY

OF THE

College of Physicians and Surgeons

COLUMBIA UNIVERSITY
N. Y.



VOL. X.

FOR THE COLLEGIATE YEAR 1904-1905

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NOTE.

These reprints of the more important studies published by the workers in this department in various journals during the collegiate year 1904-1905 are brought together in this form for the convenience in reference of those interested in these themes.

It has seemed to us that by this form of publication—first, the wider circulation which current medical journals afford, and the subsequent grouping together of reprints—the aims of such varied studies are more certainly accomplished than they would be by a special department or university publication.

The contents of previous issues may be found at the end of this volume.

The cost of reprinting and issuing these studies is defrayed by the Alumni Association of the College of Physicians and Surgeons.

T. MITCHELL PRUDDEN,

Director

DEPARTMENT OF PATHOLOGY,
COLLEGE OF PHYSICIANS AND SURGEONS,
COLUMBIA UNIVERSITY, NEW YORK,
March, 1906.

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A CONTRIBUTION TO THE PHYSIOLOGICAL DIFFERENTIATION OF PNEUMOCOCCUS AND STREPTOCOCCUS, AND TO METHODS OF STAINING CAPSULES.¹

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GENERAL CONSIDERATIONS.

Pneumococci and streptococci which do not differ in morphology from their classic types can usually be differentiated from each other and identified by their morphological characters without difficulty; but it is equally true that certain cultures of these organisms, either

¹ An abstract of this paper was read at the Chicago meeting of The Society of American Bacteriologists, December 31, 1901, and published in *Ctbl. f. Bakt.*, 1902, xxxi, 302; and in *Science*, Mar. 7, 1902, 367.

² Received for publication March 18, 1902.

at the time of their isolation or after cultivation on artificial media, approach the type of the other so closely that it may be impossible to identify them by their morphology alone. When such morphological variations occur there are no constant and distinctive cultural or pathogenic characters as yet demonstrated, which can with certainty be depended upon as distinguishing marks between these organisms.*

This lack of distinct cultural differences between pneumococci and streptococci has not infrequently led to confusion, but that uncertainty should exist and mistakes be made in identification is not surprising when one considers the characters usually depended upon to distinguish pneumococci from streptococci. Chief among these, as has just been implied, are the morphological features, which are in the case of the pneumococcus a slightly lancet or elongated variation of the more typical coccus form which is characteristic of the streptococci, the arrangement of such cocci in pairs rather than in chains, and the possession of a more or less well-defined capsule. All of these characters are subject to variation or may be absent. Compared with the morphological, the cultural characters are of minor importance, and are variable. They consist in a more watery appearance of the pneumococcus colonies on coagulated blood serum and on agar, and in the usual inability of the freshly isolated pneumococcus to develop readily or at all on gelatin at temperatures below 22° C.

The distinctness of the capsule of the pneumococcus in the body fluids of man and animals, and at times when this organism is artificially cultivated in blood serum, milk or serum agar, has really been depended upon as the chief distinguishing and diagnostic character.

During the past few years, however, from time to time, instances have been reported of distinct capsule formation by organisms which had either been previously identified as streptococcus pyogenes, or

* On this subject see Welch, *Bulletin of the Johns Hopkins Hospital*, 1892, December, p. 125 et seq.

at the time of their isolation could not be definitely identified by their discoverers as belonging to either this group or to the pneumococci, but were considered intermediate in their character.

Brief Description of Organisms Reported as Capsulated Streptococci.—

Bordet (1) working with an organism previously identified as streptococcus pyogenes described such capsule formation occurring in the peritoneal exudate of infected rabbits.

Schütz's *diplokokkus der Brustseuche der Pferde* (2), Poels and Nolen's streptococcus of contagious pneumonia of cattle (3), and especially the organism described by Bonome (4) as "*streptokokkus der Meningitis cerebro-spinalis epidemica*," may all be looked upon as organisms differentiated on insecure grounds from either pneumococcus or streptococcus. The first two of these organisms, however, are said to be decolorized by Gram's method, and as suggested by Frosch and Kolle (5) in the case of Schütz's organism, may belong to a group intermediate between Fraenkel's diplococcus and the chicken cholera group.

Tavel and Krumbein (6) describe a streptococcus with a capsule, which was isolated from a small abscess on the finger of a child. Capsules were also present in the artificial cultures, and although ordinarily remaining uncolored, could be stained by Loeffler's flagella stain. This organism was said to be differentiated from Fraenkel's diplococcus and also in general from the streptococci (pyogenes) by a rapid and rich growth on gelatin, agar, and potato. A pellicle was formed on broth. The organisms forming this pellicle possessed capsules, but those in the deeper portions of the broth generally lacked the capsule.

In 1897, Binaghi (7) described a capsulated streptococcus isolated from a guinea-pig dead of a spontaneous peribronchitis and multiple pulmonary abscesses. In the pus were found some diplococci and short chains (4 to 6) surrounded by a capsule, which could be made evident by staining with carbol fuchsin. This organism he proposes to call *Streptococcus capsulatus*.

Le Roy des Barres and Weinberg (8) in 1899 published an account of a streptococcus with a capsule. This was isolated from a man who had apparently been infected from a horse which had died of an acute intestinal disorder. The patient neglected the infection and died. Diplococci and short chains furnished with a capsule were found in the subcutaneous tissue at the area of infection. The blood, liver and spleen also contained these organisms. The capsule in all the preparations remained uncolored, but the authors say that its existence was not to be doubted.

Ascitic broth inoculated from the peritoneal exudate of a rabbit dying from the infection gave streptococci in extremely long chains and surrounded by capsules. These were not so distinct as in the case of the organisms in the original smear preparations. Nothing notable was observed in the cultural characters. All fluid media (bouillon, milk and ascitic broth) were said to be strongly acid after twenty-four hours. These authors report that Achard and Marmorek have assured them that they have seen capsulated streptococci, and that Marmorek showed them some preparations in which one of his streptococci presented the same characters as that isolated by them.

Although Le Roy des Barres and Weinberg have used the term encapsulated, they believe that it would perhaps be more prudent to call their organism *streptocoque auréolé*, since they were not able to put this capsule definitely in evidence by staining it.

Howard and Perkins (9) have lately described an organism, probably of the foregoing type, which was present in a tubo-ovarian abscess and in the peritoneal exudate, the blood and some of the organs of a woman dying in the Lakeside Hospital, Cleveland, Ohio. The organisms were biscuit-shaped cocci in pairs, usually arranged in chains of four, six, eight or twenty elements, and surrounded by a wide and sharply staining capsule. In the artificial cultures special capsule stains, it was noted, failed to stain any definite area, but numerous small deeply stained granules were to be seen within the halo, especially near its outer border. Capsules in litmus-milk could be sharply stained. Howard and Perkins propose for the group composed of the streptococci of Bonome, Bin-
aghi, and their own organism, the name *Streptococcus mucosus*.⁴

Reference to the original descriptions of these various capsulated streptococci will show that, with the exception of a rather poorly staining capsule, the majority of these organisms are separated from the typical streptococcus pyogenes or from the pneumococcus by exceedingly slight and unstable morphological and cultural characters. The same is true of the difference observed in their pathogenic action in animals.

There are occasions, then, both within the animal body and in artificial cultivations when it is practically impossible to distinguish definitely between some races of pneumococci and races of strepto-

⁴ Through the kindness of Dr. Perkins, I have had an opportunity of studying the organism of Howard and Perkins. It ferments inulin and in most characters shows a closer affinity with pneumococci, than with true streptococci.

cocci. This difficulty is especially heightened when the pneumococcus has become non-virulent, and at the same time no very typical morphology or capsule formation is to be determined and a tendency to chain formation is marked. Cultures of pneumococci in such condition have come under my notice and were not readily to be distinguished morphologically from streptococcus cultures.

Under these circumstances there has been up to the present time no means of determining morphological or cultural differences by which these organisms could be definitely distinguished. The only recourse is the tedious attempt to revivify the pathogenicity by introduction of the culture into the most susceptible animals and to thus bring again into prominence the lancet-shaped diplococcus type, surrounded by the capsules which characterize the pneumococcus in animal fluids.

My work during the past two years with many cultures of pneumococci and streptococci from various sources has shown certain physiological differences in these organisms, which are, so far as I am able to determine, fixed characters, and have thus far proved unfailing means of distinguishing pneumococci from any streptococcus pyogenes met with. It is, of course, understood that the streptococci here referred to are organisms which would ordinarily be classed as streptococcus pyogenes (*longus*, *brevis*, *conglomeratus*), *erysipelatos*, or *scarlatinæ*.

In the course of these experiments I have also been led to the development of simple staining methods for capsules, and to the application of special means, cultural and otherwise, of demonstrating the presence of capsules on both pneumococci and streptococci.

It is the object of the present paper to set forth as briefly as may be these differential and capsule experiments.

THE PHYSIOLOGICAL DIFFERENTIATION OF PNEUMOCOCCUS FROM STREPTOCOCCUS.

PRELIMINARY OBSERVATIONS LEADING TO THE EXPERIMENTS IN DIFFERENTIATION.—My attention was first called to the possibility of a cultural difference between pneumococci and streptococci early in 1900. At

this time I had prepared some serum broth, composed of two parts of broth and one part of an inflammatory exudate from the human pleural cavity. This fluid had originally been rich in cells and was fairly thick, the proteid content being high. Attempts to sterilize at 68° C. had resulted in the solidification or gelatinization of the mixture, and even at 60° C. this was found to take place. The medium was finally sterilized in a fluid condition at 55° C. On March 31, I had occasion to use this medium for the cultivation of a pneumococcus which on March 24 had been isolated from some exudate coming from a case of meningitis in a child following pneumonia. In the serum-broth, after 24 hours' growth at 37° C., the organism brought about a nearly solid coagulum of a yellowish-white color. The culture was examined morphologically by the Welch capsule stain, and was found to be a pure culture of pneumococcus with clearly stained capsules. Subcultures could not be obtained from this tube after 48 hours at 37° C.

The reaction of the uninoculated serum-broth was tested and found to be 0.75% acid, phenolphthalein being the indicator. The coagulated medium containing the pneumococci reacted 2.5% acid. The coagulum dissolved upon the neutralization of the acid.

Shortly subsequent to this, tubes of this same serum-broth were inoculated with two cultures of streptococci. One of the latter was in use in immunizing experiments at the Research Laboratory of the New York Health Department, and the other was isolated by me from the exudate of an empyaema in a child. These, it was noted, did not coagulate the medium even after some days' growth at 37° C.

I was impressed with this difference in coagulative action of the pneumococcus and streptococci in this medium and, realizing that it might indicate more than a temporary difference in the metabolism of these organisms and might thus be of diagnostic value, I made further tests and endeavored to analyze the phenomenon. This, it seemed, might depend upon at least two things—either it might be due to an acid formed by the pneumococcus in the presence of some fermentable substance (probably carbohydrate) not available for the streptococci, or it might be due to an acid produced by the pneumococcus quite independent of a carbohydrate in its nutrient surroundings. That acid was formed and that the precipitate was due in large part to this was indicated by the titration and by the resolving of the coagulum upon the addition of an alkali.

What the fermentable substance was, if there really was one present, which is not unlikely, was not determined.

The normal amount of glucose in fresh beef serum, when the serum is mixed with broth in the proportion of 1 to 2 and the glucose fermented by organisms known to act upon it, such as *B. coli communis* and *B. typhosus*, does not lead to such a coagulation, neither did these organisms give a coagulum when grown in the inflammatory serum broth. It seemed, therefore, that this substance, if it were a carbohydrate, must be one of the less readily fermented, such, for instance, as glycogen; or it might be that some glucoprotein or possibly nucleoprotein, which could be broken up by the pneumococcus, was present in excess of the usual amount. This latter supposition is not unreasonable, as the cell content of the serum was large.

It did not seem probable that glycogen, even if originally present, could long remain in such a serum unchanged by the diastasic and maltasic ferments, which are normally present in blood and such exudates. Experiments were, however, carried on with various carbohydrates, with the hope of thus throwing some light on the problem, and they have led to such interesting and, it is hoped, valuable results, that they form an important part of the present communication.

No attempt has as yet been made to investigate the glucoproteins and nucleoproteins.

CULTURE EXPERIMENTS WITH SERUM-BROTH MEDIA, STERILIZED AT 65°-70° C.—In the first attempt to devise a differential culture medium, fresh beef-serum was diluted with sugar-free broth, reacting 1% acid⁵ to phenolphthalein, in the proportion of two parts of broth to one part of serum. This was then divided into separate portions, one of which was left plain, and to the others were added respectively dextrose 1%, lactose 1%, saccharose 1%, dextrin 1% and starch $\frac{2}{3}$ %.

These media were sterilized at 65°-68° C. for one hour on six consecutive days. They were inoculated with two cultures of pneumococci and four of streptococci.

The dextrose, lactose, saccharose and dextrin media were found to be fermented by the streptococci as well as by the pneumococci, with the formation of a solid, yellowish-white coagulum, due to the resulting acid.

⁵ This broth was neutral to litmus.

The starch medium yielded the most satisfactory differential results. This medium was rapidly fermented by the pneumococci, but was noted only after 13 days' growth at 37° C. as becoming gelatinous from the action of the streptococcus cultures. The plain serum was not coagulated by either organism. Anaërobic cultivations were made. The results were practically the same as given by aërobic growth with the exception of the coagulation of the plain serum-broth by the pneumococcus.

The results indicated by the starch medium were promising. They showed that these starch preparations could be readily fermented by pneumococci with rapid acid formation, and that the streptococci, on the other hand, although they developed in the medium, could not avail themselves of the starch, at least with ease, for it was generally a matter of many days before even a gelatinization of the medium resulted.

As there was, as determined by the iodine test and by the use of Fehling's solution, a marked conversion of the starch during the preparation of the media, and by this the introduction of complicating factors into the tests, changes were made in the mode of preparing the medium.

CULTURE EXPERIMENTS WITH SERUM-WATER MEDIA, STERILIZED AT 65°-70° C.—Distilled water was substituted for the broth in these experiments so as to exclude any hydrolization in the presence of acids and salts, such as are usually present in broth, during the preparation of the starch.

Starch water was prepared by adding 4 grams of powdered starch to 400 cc. of water, boiling for one-half hour, and allowing to stand over night. In the morning a clear fluid could be obtained by pipetting off the water from which the starch particles had settled to the bottom of the flask. This was then added to serum in the proportions of serum 1 part, starch water 2 parts, and sterilized at 68° C. for 1 hour on 6 consecutive days. A medium containing glycogen 1% was also made.

A test of these media showed that in both of them the pneumococcus not only grew readily but induced a coagulum in the glycogen as well as in the starch medium, and this often within 24 hours, at 37° C. The streptococcus cultures grew well, but, as in the first experiment, did not coagulate the starch medium readily, nor bring about coagulation of the glycogen serum, except after many days, and in some cases apparently were not able to do this at all.

Having proved by this preliminary experiment that growth of pneumococci and streptococci would take place in serum diluted with distilled water, it was possible thereafter to avoid the complicating factors incident to the use of broth.

Besides the starch and glycogen media already mentioned, tests were made also with aqueous serum media plus dextrose, lactose, galactose, maltose, saccharose. All of these mono- di- and polysaccharids were readily and rapidly used by the pneumococci, and gave rise in all cases to acid sufficient to cause the coagulation of the albuminous material in the serum. The streptococcus cultures as a rule readily fermented dextrose, galactose, and the maltose (commercial specimen), less readily lactose and saccharose; but starch and glycogen, as noted above, if changed at all, were usually only sufficiently affected by the streptococci to give rise to coagulation after many days and after some evaporation from remaining at 37° C.

These results indicated a marked difference in degree, but not necessarily in kind, between the fermentative power of the pneumococcus and streptococcus cultures as a class. No perfectly distinct differentiation, however, had so far been obtained between the pneumococcus and all of the streptococcus cultures tested.

CULTURE EXPERIMENTS WITH ALKALINE SERUM-WATER MEDIA, STERILIZED AT 100° C.—It was realized that diastasic action going on during the preparation of the media might affect the result of these experiments. Changes might thus be brought about either by the diastase and maltase (glucose) and glycolytic ferments of the

blood or by the action of contaminating bacteria or their accompanying ferments during the long process of low temperature sterilization at 65°-70° C.

The diastasic action of beef serum, as was demonstrated in some experiments, in such mixtures as those used by me, was exceedingly rapid and well marked, as was also the action of maltase. Starch serum mixtures rapidly failed to give the blue reaction with iodine, and after standing for a few hours or even less at the room temperature failed to give even the brown reaction indicating the presence of erythrodextrin. Those heated to 65°-70° C. failed in these reactions even sooner. This change must be equally true in the case of glycogen, and indicated that our results were not necessarily due to the action of our organism on pure starch or glycogen, but probably on some product of the fermentation of these substances by the enzymes of the blood or by bacteria present during their preparation. Aside from these complications, low temperature sterilizations are at best tedious and unsatisfactory. An attempt was therefore made to eliminate these factors of uncertainty in the preparation of the media.

The first experiment was made by adding small amounts of alkali to the serum-water media, made as above by adding 1 part of beef-serum to 2 parts of distilled water, with the object of converting the proteids into alkali albuminates which could be boiled without coagulating.

By the addition of 1 cc. *n*/1 NaOH to the serum water, a perfectly clear fluid resulted which could be sterilized at 100° C., and remain uncoagulated and clear. One-half per cent *n*/1 NaOH also gave a non-coagulable medium, but was not quite as clear after boiling as the 1%. Such media are, however, open to the objection that the sugars or starches may be changed when boiled in the presence of an alkali. Gradual reductions of the alkali content were therefore practised and it was eventually found that no added alkali was needed to prevent coagulation in mixtures in the proportion of one part of ox-serum to two parts of distilled water. Such a medium,

although becoming opalescent at boiling temperature, remains perfectly fluid, and is available as a nutrient base for such sugar tests.

Before these last results were obtained, tests were made with the alkaline media plus some of the sugars, and also with the plain alkaline medium. The sugar tests showed nothing especially worth noting except that a longer interval elapsed before the coagulation of the albumins took place, this being due to the additional alkali which had to be neutralized.

The chief point of interest and value developed in connection with the use of a medium without sugar but which had been rendered slightly alkaline by the addition of 0.2% of $n/1$ NaOH. In this medium the pneumococcus cultures invariably gave rise to an opalescence, a gradual whitening and final gelatinization and coagulation. The amount of alkali was afterwards reduced to 0.1% $n/1$ NaOH, and this medium was found to give rise when grown in the incubator at 37° C. to the same opalescence and whitening after about 48 hours, with a firm coagulation after some days, depending upon the culture tested. As may be seen in the appended Table I, no streptococcus cultures bring about visible changes in this medium.

We thus have in this alkaline medium, made of one part of ox-serum and two parts of distilled water plus 0.1% $n/1$ NaOH and sterilized at 100° C., a means of differentiating the pneumococcus from various cultures of streptococci, and so far as my experience goes from all. The behavior of the pneumococcus in this medium seems to indicate a fundamental difference in its metabolism from that of the streptococci. Upon what metabolic process and nutrient ingredients this formation of acid depends has not as yet been determined.

TABLE 1.

TEST OF PNEUMOCOCCI AND STREPTOCOCCI IN THE ALKALINE SERUM-WATER MEDIUM. 0.1% N/1 NaOH.

DAYS.	PNEUMOCOCCUS CULTURES.				STREPTOCOCCUS CULTURES.
	V	VI	VII	VIII	IX
1
2	Becoming opalescent.	Becoming opalescent.	..	Becoming opalescent.	..
3	White.	White.	Becoming opalescent.	White.	..
4	Gelatinous.	Gelatinous.	White.	Gelatinous.	Becoming opalescent.
5	Semisolid.	Semisolid.	"	Semisolid.	White
6	"	"	"	"	"
7	"	"	"	"	"
8	+	+	Gelatinous.	+	Gelatinous.
9	+	..	Semisolid.
10	+
20

Twenty-eight cultures were tested; none of these brought about any visible change in the medium.

+ Indicates a solid coagulum.

CULTURE EXPERIMENTS WITH SERUM-WATER MEDIA, STERILIZED AT 100° C.—After it had been observed that a mixture of one part of beef-serum and two parts of distilled water could be boiled and thus sterilized without precipitating the albuminous materials present, media were prepared in this manner, containing 1% of various carbohydrates—thus, dextrose, galactose, maltose, lactose, saccharose, dextrin, starch, glycogen, and inulin. These carbohydrates, with the exception of the maltose, which was a fair commercial sample, were of high purity, the galactose, lactose and glycogen^{*} having been especially prepared and tested for this work. Such precautions, it need hardly be remarked, are absolutely essential to the success of these experiments. Samples of glycogen made by manufacturers, and bought in the open market, gave results totally at variance with those obtained when the specially prepared glycogen was tested. More readily fermentable carbohydrates were always found to be present with the glycogen in manufacturers' samples.

This series of experiments with the carbohydrates showed that the monosaccharids—dextrose, levulose, galactose—were fermented readily by practically all streptococci as well as by the pneumococci. This is true also of the disaccharids—maltose, lactose, saccharose—as regards the streptococci as a class, but the development of the coagulum may be much slower than in the case of the monosaccharids, and with certain cultures of streptococci it may be will not occur at all.[†] In the case of the polysaccharids—dextrin, starch and glycogen—coagulation usually takes place only after many days in streptococcus cultures, and in some cultures apparently does not occur. The non-coagulation is more frequent with glycogen than with starch. Inulin, however, is not fermented by any of the streptococci, although it is readily used by the pneumococci in such serum media. Growing in such an inulin medium, the pneumococcus rapidly gives rise to acid, which leads to the formation of a solid white coagulum which is

^{*}These samples of glycogen, lactose and galactose were especially prepared for me by Dr. A. N. Richards, to whom my sincere thanks are due.

[†]This subject will be discussed more fully in a later paper on the fermentative power of the streptococcus group.

usually complete within 48 hours. By the use of this medium a rapid and constant differentiation can be obtained between pneumococci and the streptococcus pyogenes group.

The differences in behavior of the streptococci in inulin from their behavior in starch and glycogen—the constancy of the non-coagulation—may in part be accounted for by the character of the inulin itself. Inulin is obtained chemically pure and is therefore free from contamination with more readily fermentable carbohydrates. Furthermore, it apparently is not hydrolized during boiling in such a serum medium, nor is it affected by the diastasic ferment present in the blood serum. Glycogen, on the other hand, is only obtained in a comparatively pure state with difficulty, is probably hydrolized to some extent by boiling after it is added to the medium, and is readily acted upon by the diastase of the blood, and subsequently by the maltase, if the preparation be not rapidly carried on or the serum-water heated previous to its addition. These facts may account for some discrepant results, although some are most likely due to differences in action of the streptococci, and may indicate fundamental differences in their physiology.

Preparation of inulin serum-water medium.—The inulin medium is satisfactorily prepared as follows: To one part of fresh, clear beef-serum is added two parts of distilled water, and to this mixture is added 1% of pure inulin. The inulin goes into solution slowly in the cold, and the mixture may be warmed to 55°-60° C. to hasten this solution.* As soon as the inulin is dissolved, the medium should be tubed, and sterilized immediately at 100° C. for ten minutes.* This sterilization is repeated on the two following days. The medium prepared in this way usually becomes only slightly turbid, and no unfavorable changes occur in it.

While this is a favorable method of preparing the inulin medium and no hydrolization or change of the inulin is effected by the action

* The inulin may be dissolved in the distilled water before it is added to the serum.

* Very resistant spores are at times present in inulin. The usual three heatings are then not sufficient, and the medium should be sterilized in the autoclave, at 10 to 15 pounds for 15 minutes.

of the enzymes of the serum, it may also be prepared by first heating the serum-water mixture to 100° C. for some minutes and then adding the inulin. This method should always be followed in preparing other carbohydrate serum-water media. Litmus solution (Merck's highly purified litmus, 5% solution in distilled water) may be added to this media in the proportion of 1%, and changes in reaction thus detected by the color change.

Since sera, even from the same species of animal, differ at times in their initial degree of alkalinity and salt content, specimens are met with, though rarely, which are of so low an alkalinity or have so high a salt content that media made from them are extremely white and opaque, and near the point of heat coagulation. Such media should not be used.

The results of tests of various pneumococcus and streptococcus cultures in 1% inulin serum are shown in Table II. It will be seen that the pneumococcus cultures tested usually coagulated the serum within 48 hours, while the streptococcus cultures, 28 of which were tested, were without effect, although all of them grew at least feebly in the medium.

SUMMARY OF CULTURE EXPERIMENTS.—It is plain from the foregoing experiments that the pneumococcus has a remarkable ability to utilize carbohydrates in its metabolic processes—all mono-, di- and polysaccharids that were tested being rapidly fermented by it as demonstrated by the production of acid. Further than this, the pneumococcus can produce sufficient acid to give rise to a coagulum in a serum medium which is sugar free, or which at least does not contain enough fermentable saccharine matter for the production of appreciable acid by such well-known fermenting organisms as the colon bacillus and the typhoid bacillus, which readily give rise to acid in the presence of glucose.

The streptococci, on the other hand, have apparently more limited and certainly less active fermentative powers. While they usually ferment the monosaccharids and in most instances the disaccharids

TABLE II.
TEST OF PNEUMOCOCCI AND STREPTOCOCCI IN THE INULIN SERUM-WATER MEDIUM.

DAYS.	PNEUMOCOCCUS CULTURES.								STREPTOCOCCUS CULTURES.
	V	VI	VII	VIII	IX	X	XI	XII	XIII
1	+	+	+	+	+	..
2	+	+	+
3	Gelatinous.
4	"
5	"
6	"
7	"
8	+
9
10
20

+ Indicates a solid coagulum.

Twenty-eight cultures were tested; none of these brought about any visible change in the medium.

rapidly and with ease, the polysaccharids they either do not ferment at all or very slowly. Glycogen and starch are fermented only by certain species or races of the streptococci; inulin is fermented by none so far tested. Furthermore, an appreciable amount of acid is not produced in sugar-free serum media by any streptococcus pyogenes so far as our experience goes.

We may assume, therefore—although one hesitates to dogmatize even after extended experiments on a group of organisms whose physiology is so little understood—that there are distinct differences in respect to the physiological processes of these two forms, or to speak more correctly, of the pneumococcus on one side and the streptococcus group on the other.

The relationship of these organisms may be compared to that which exists between typhoid bacilli and the bacilli of the Gärtner-colon group. The pneumococci resemble the typhoid bacilli in the distinctness and apparent permanence of their cultural characters, while the streptococci seem to form a group which resembles the Gärtner-colon group in presenting grades of fermentative activity, and especially as this activity is displayed by the streptococci in such aqueous serum media as those used in these experiments. In making this comparison it is recognized that the pneumococcus is to be known by positive cultural characters as shown by its ability to ferment the various mono-, di- and polysaccharids, while the typhoid bacilli are chiefly distinguished in comparison with the members of the Gärtner-colon group by the absence of certain physiological activities, such as gas and indol production and the coagulation of milk. Still by this comparison it is simply desired to emphasize the co-extensiveness of the characters, as shown by these experiments, of the organisms commonly recognized as pneumococci, as compared with the variety or variability of the characters displayed by organisms which would ordinarily be classed as streptococci, and probably as streptococcus pyogenes.

EXPERIMENTS ON THE DEMONSTRATION AND STAINING OF CAPSULES
ON PNEUMOCOCCI AND STREPTOCOCCI.

Welch's stain for capsules was the stain constantly used in the earlier of these experiments, and as a rule gave excellent results. Certain conditions, however, at times arose in some of the cultures which interfered with the success of this stain, and I was thus led to seek other methods of staining and of demonstrating the capsules on pneumococci.

NEW METHODS DEvised FOR STAINING CAPSULES.—After many experiments it was found that a very satisfactory and rapid staining of the pneumococcus capsules could be effected by the following method:

Potassium carbonate method.—This method consists in using as a dye a half-saturated aqueous solution of gentian violet.¹⁰ This is applied for a few seconds to the cover-glass preparation, which has previously been allowed to dry in the air, and has been fixed by heat in the usual manner. No water must be used in diluting and spreading the organisms on the cover-glass. If the organisms come from a solid medium or fluid medium other than fluid sera, some other diluting and spreading fluid such as will be described later must be used. The dye is washed off with a one-fourth per cent (0.25%) aqueous solution of potassium carbonate and the specimen is studied in this fluid. The cover-glass may be sealed on the slide by rimming with vaseline, and evaporation thus prevented. This stain gives remarkable results when used on pneumococci either from fluid or hardened serum media or from the body fluids. (See Figs. 1, 3, 4.) The capsules are large, prominent, and are either stained throughout, or their periphery appears as a dark line or layer, the part next to the deeply stained diplococci being less intensely stained than the periphery. This appearance may be due in part to a precipitation of the dye on the exterior of the capsules by the potassium carbonate.

¹⁰ Gentian violet in substance is added in excess to distilled water and allowed to dissolve to its full extent. The solution is then filtered, and diluted to twice its volume.

Very fair capsule stains may also be obtained by simply staining for about thirty seconds with the ordinary aqueous gentian violet solution (5 cc. sat. alc. sol. to 95 cc. of distilled water) and then washing with a 1% potassium carbonate solution and studying the specimen in the fluid.

Preparations stained by the potassium carbonate method may at times give satisfactory results when dried and mounted in balsam (preferably gum Damar), but this is not the rule.

The following method, however, is eminently satisfactory when it is desired to mount specimens of pneumococci with capsules in balsam.

Copper sulphate method.—In this method a 5% or 10% aqueous solution of gentian violet or fuchsin (5 cc. sat. alc. sol. to 95 cc. distilled water) is placed on the preparation, which has been prepared as previously indicated. The dye is allowed to steam for a few seconds, by gradually passing the cover-glass downwards through the flame several times. After this the staining fluid is washed off with a 20% solution of copper sulphate. This solution may vary from 10% to saturation, but the medium strength, 20%, is generally satisfactory. After washing with copper sulphate, the preparation is dried between filter papers and when thoroughly dry mounted in balsam. The capsules remain of normal size, stained, and distinct. (Figure 2.)

OBSERVATIONS AND EXPERIMENTS ON MEDIA AND CONDITIONS FAVORABLE TO CAPSULE DEVELOPMENT AND STAINING.—The most favorable conditions known for the development of the pneumococcus capsule are found in body fluids of man and animals suffering from an infection with this organism. For instance, capsules may be demonstrated with ease by the usual methods in the blood, serum and inflammatory exudates of the infected rabbit which is, among test animals, one of the most favorable for these experiments. Capsules may be equally well marked in the fresh sputum of pneumonia patients, especially in the early stages of the disease, and in the exudates accompanying such pneumococcus infections as meningitis, otitis media, and empyaema. In sputum and the exudates of these

various localized infections the organisms are, however, frequently degenerated or under chemical conditions unfavorable for capsule staining, and satisfactory results are then not easily to be obtained. The same is true of the scrapings from lungs of patients dead of pneumonia, often even in the stage of red hepatization. Under these conditions a longer exposure to the staining reagent is necessary, before the organisms and capsules are brought into prominence, and even then the results in nowise compare to those obtained with organisms in fresh sputum or the body fluids of such an animal as the rabbit.

It was shown by Ortman (10), as early as 1888, that outside of the animal or human body pneumococci regularly developed capsules when cultivated in blood serum. Welch¹¹ in 1892 and Paulsen (11) in 1893 called attention to the development of capsules on these organisms in milk, and Schmidt (12) showed that sputum media favored the formation or preservation of pneumococcus capsules. Schabad (13) made a similar observation in 1896 in regard to blood agar. Frosch and Kolle (14) refer to the demonstration of capsules on pneumococci cultivated in Guarnieri's medium, and rather indefinite statements in regard to capsules on pneumococci coming from broth and other cultivations may be found in various articles on the pneumococci.

In my own work, most of these experiments have been repeated and the results confirmed. In the serum media of various composition used during the experiments on the physiological differentiation of pneumococci and streptococci, the pneumococci not only grew readily but developed distinct and well-marked capsules. This capsule formation seems to be independent of the length of time the organism has been under artificial cultivation. One of the most favorable media of all for the development of capsules was that made of 1% starch-bouillon and serum—serum 1 part, bouillon plus 1% starch 2 parts—and sterilized at 65°-70° C. In this medium, according to my experience, capsules are developed with great regu-

¹¹ loc. cit.

larity and may be stained without difficulty. The same is true of pneumococci grown on Loeffler's coagulated blood serum, or on coagulated serum without glucose; and these organisms may be prepared for staining by simply spreading them on the cover-glass in some of the condensation water from the serum tube. This use of condensation water may also be successful in the case of organisms growing on plain or glycerine agar, but the most successful method to preserve or perhaps to accentuate the capsule on pneumococci or streptococci coming from artificial media, other than fluid serum media, is to use a drop of serum as the diluting and spreading fluid for the cover-glass preparations. Some of the carbohydrate serum mixtures, such as the starch or glycogen media and the alkaline media, often serve this purpose better than unmodified or fresh serum.

My first experiments in this direction were with the serum starch mixtures used for cultural purposes. It occurred to me that such a fluid in which the pneumococcus capsules were always present and demonstrable by staining might serve to preserve or even develop them on organisms when these were transferred to a drop of the fluid from various artificial media, such as broth, agar, etc. This is successful with pneumococcus nearly without exception, and the presence of a capsule bears little relation to the time the organism has been cultivated artificially, or the medium from which it comes. It is well, however, to have fresh cultures, say usually not over 24 hours old.

It is to be noted that such a fixing medium does not bring the capsule into prominence by simply serving as a deeply stained background and leaving unstained around the organisms the so-called "retraction zone." By the potassium carbonate method the diluting medium often remains practically unstained, while the capsule stands out plainly, either stained definitely throughout, or with a distinct peripheral line or layer which shows to perfection when the organism is free from surrounding detritus. In the copper sulphate method the capsules stain uniformly while the field may or may not be free from detritus.

In smears from the serum of infected animals, *i. e.*, rabbits with pneumococcus, the potassium carbonate method leaves everything practically unstained but the organisms and their capsules. Of course, if blood cells are present they take the stain to some extent.

With the copper sulphate method the serum is stained, but is contracted into threads and masses, and does not give a uniform background. (Figure 2.)

The following experiment made with a bacillus isolated in company with a streptococcus from a case of endocarditis, but probably having no etiological significance, shows the advantage in staining capsules of preparations made with serum or serum mixtures. A smear was made directly on a cover-slip from an agar culture, no diluting fluid being used. By the potassium carbonate method a small capsule was demonstrated. When, however, it was mixed on the cover-glass with some serum (glycogen serum) and stained by the same method, very large capsules were found. In some instances the bacilli could be seen dislodged from their central position in the capsules and sticking half way out of them; in other instances the bacilli were seen entirely free and lying naked alongside of the empty capsules. The photograph of this specimen was made from a preparation stained with aqueous gentian violet, washed with 0.25% sol. of potassic carbonate, dried and mounted in balsam. (Figure 12.)

DEMONSTRATION AND STAINING OF CAPSULES ON STREPTOCOCCI.—
In determining the growth of the streptococci in the various experimental media, the cover-glass preparations were often stained by the same method (K_2CO_3) as that used to demonstrate the capsule of the pneumococcus. In this way the fact was brought to light that the streptococci of the cultures tested were possessed of capsules, which stained or were made visible by these methods. This was especially true of cultures in sugar serum media, and in the more alkaline serum media. In some cultures the capsules were quite as sharp and as well-defined as those of the pneumococcus; in others

they were less well marked, and in some appeared as if in a semi-fluid state and on the point of dissolving. In these last, when several organisms were massed together, their capsules seemed to coalesce. This, however, may occur with pneumococcus capsules, though it is by no means so frequent.

Source of the Streptococcus Cultures Used in these Experiments.—The source of the various streptococci which have been used in the experiments will be of interest in this connection, since, as was noted in the earlier part of this paper, the presence of capsules on certain streptococci has been looked upon as a character sufficient to place them in separate species.

The cultures enumerated are those which have been used in all of the cultural and capsule tests.

No. 1. Isolated from milk, May, 1898. Has been cultivated on artificial media for many months.

No. 2. Isolated from human throat (diphtheria suspected) November 18, 1898. Has been long on artificial media.

No. 3. Isolated from an abscess of a horse following subcutaneous injection of diphtheria toxin (filtered), May 17, 1900. Some months at least on artificial media.

No. 4. Isolated from another antitoxin horse. Nasal discharge due to disease of nasal sinus, probably necrosis of bone. November, 1901.¹¹

No. 5. Isolated from a case of suppurative pyelophlebitis in man. This organism accompanied a bacillus which was a strict anaërobe.¹²

No. 7. From a case of appendicitis.

No. 8. Culture marked "M" from the Research Laboratory, Department of Health, N. Y. Isolated from a case of erysipelas.

No. 9. Same culture as "8," but grown for many months as a separate culture under slightly different cultural conditions.

No. 10. Said to have come from a case of scarlet fever. Research Laboratory, Department of Health, N. Y. Marked "B."

No. 11. Present in pure culture in the urine of a man suffering from a marked cystitis. Patient gave history of chronic pyuria. Capsules on streptococci in the original urine smears.

¹¹ I am indebted to Dr. Theobald Smith for these four cultures. They were labelled I, IV, VII, X in the order enumerated in the text.

¹² See Norris, *Journal of Medical Research*, 1901, I, 97. I am also indebted to Dr. Norris for culture 7.

No. 12. Rapid ascending cellulitis of leg. Tissues after 24 hours appeared gangrenous. Patient showed marked signs of sepsis, but eventually recovered.

No. 13. Isolated from the heart blood of a patient showing lesions of endocarditis.

No. 14. Isolated from human uterus. Puerperal sepsis.¹⁴

These cultures from such widely different sources present the usual characters ascribed to streptococcus pyogenes, and so far as I have studied them, are not to be differentiated from one another by the ordinary cultural tests. Morphologically they do not present more than the ordinary slight differences which may be probably attributed to slight variations in physical and chemical environment, rather than to constant inherent differences in the organisms themselves.

All of them coagulate milk after a varying number of days, and grow in gelatin at the room temperature. Nos. 8, 9 and 10 developed tardily in gelatin, did not extend along the puncture to the surface of the medium, but grew along the lower half of the line of inoculation. All of the other cultures grew readily in gelatin, some of them spreading slightly away from the point of inoculation on the surface of the medium.

The broth cultures were not especially noteworthy, except in the different behavior of the same organism when tested in broth containing sugars, and in sugar-free broth. Either flocculent growth or uniform clouding may occur according to the character of the broth in which cultivations are made. Uniformly clouding non-sugar broth, they may present flocculi in one or more of the sugar broths, or vice versa, according to the culture tested. These characters seem to be independent of the acid produced in the medium. I note, for example, organism "8," *glucose broth*: uniform clouding, dense; *plain broth*: flocculi, medium amount of growth. Organism "12," *plain broth*: flocculi small, practically uniform clouding; *glucose broth*: flocculi large, plentiful sticky growth, organisms in very long chains.

All of the streptococci from the above described sources have been found to possess capsules which become apparent under various conditions when stained by the methods mentioned. Streptococcus No. 11 had a well-marked and easily stained capsule in the original urine smear preparations, and also in some of the artificial cultiva-

¹⁴ See Wadsworth, *American Journal of Obstetrics*, 1901, XLIII, No. 4.

tions, especially that on glycerine agar, and on serum agar. The same is true of streptococcus No. 7.

The photographs of most of the preparations were taken after the organisms had been cultivated for many generations on artificial media. As a rule, the best examples were given by cultures on ascitic serum agar. My best results with streptococci were obtained with organisms coming from serum (ascitic) agar, and diluted on the cover-glass in a drop of glycogen serum mixture which had been sterilized at 68° C., and had undergone evaporation to about one-half of its original volume." It happened that the chemical and physical condition of this mixture was exceedingly favorable to capsule preservation. Most of the photographs of streptococci were taken from specimens prepared in this way, dried in the air, fixed in the flame, and stained by the method in which a half-saturated aqueous solution of gentian violet and 0.25% potassium carbonate solution are used. Fine clear specimens may often be obtained in this way, when not a trace of capsule can be demonstrated on the organism in smears direct from fluid or other artificial media.

These experiments, in which it has been possible to demonstrate capsules in all of the cultures of streptococci tested and this often after long cultivation on artificial media, suggest that to place streptococci which have been found by the usual methods possessed of capsules, in a different specific group from the ordinary streptococci pyogenes is not warranted. And especially is this true if this character is the sole or major distinguishing feature.

In the present or immediately preceding history of such cultures, conditions favoring the development of capsules may have existed and thus brought the capsule formation to a maximum. Such conditions may have been found in the animal or human host, or as in the case examined by me, in such a medium as an albuminous purulent urine. Organisms coming from such sources, and cultivated on artificial media might well display this character for some time sub-

¹⁵ No attempt has as yet been made to reproduce this medium.

sequently more highly developed than it is among their fellows which had not had the same previous history.

I have noticed that pneumococci, which it is well known have particularly well-marked capsules in rabbit blood or serum, have this character, in some instances at least, to a less degree in the serum of guinea-pigs which have succumbed to the pneumococcus infection.

It seems more advisable, therefore, in the present state of our knowledge, to look upon capsule formation as general among streptococci, and of no absolute diagnostic or differential significance. It is also fair to conclude that unless there are cultural differences accompanying this marked development of the capsule on organisms suspected of being streptococci, that such organisms should not be placed in a separate specific group.

GENERAL SUMMARY AND CONCLUSIONS.

By morphological examination and with current cultural methods a clear differentiation cannot always be made between pneumococci and streptococci. The chief differential character usually depended upon is the capsule of the pneumococcus. Well-marked capsules, however, may occur on organisms which have with reason been classified as streptococci. On the other hand, capsules may not be demonstrable on pneumococci by the usual methods, especially when growing on artificial culture media.

The usual cultural characters and reactions are at best not diagnostic, and are subject to variations which may render them useless as evidence of specific difference.

The experiments recorded in this paper, however, afford some evidence that there are well-marked differences between the metabolic activities of pneumococci and streptococci, which may prove useful in the differentiation of these organisms. These differences in metabolism become apparent when the pneumococci and streptococci are cultivated in an alkaline serum medium, or in a serum medium to which the carbohydrate, inulin, has been added.

Pneumococci slowly produce acid in the alkaline serum.

In the inulin media they ferment the inulin and thus rapidly give rise to acid. Streptococci do not form appreciable acid in either of these media, nor do they ferment the inulin.

The differences between the metabolism of pneumococci and streptococci are indicated by visible changes in the media. Thus the alkaline serum and the inulin serum are coagulated by the acid formed during the growth of the pneumococci. This coagulation is rapid in the inulin serum medium, slower in the alkaline serum medium. The streptococci, on the other hand, do not bring about a coagulation of these media.

We have, therefore, in either of these two media, the alkaline or the inulin, so far as our experience goes, a definite means of differentiating pneumococci from streptococci.

Starch and glycogen media, prepared in the same manner as the inulin medium, are coagulated by pneumococci and by some at least of the streptococci. With the streptococci the coagulation, if it occurs at all, is usually long delayed. It may be that some or all of the streptococci do not ferment pure starch and glycogen.

Lactose, saccharose and maltose are fermented by pneumococci with the production of acid, thus giving rise to acid coagula in media containing serum. Certain members, though probably not all, of the *Streptococcus pyogenes* group ferment these disaccharids—lactose, saccharose, maltose—hence such sugars are not available in media used to differentiate pneumococci from streptococci as a group.

Monosaccharids—dextrose, galactose, possibly all monosaccharids—are fermented by pneumococcus and the various members (probably all) of the streptococcus group. Serum media containing these sugars are rapidly coagulated by the resulting acid.

In serum media, especially starch-bouillon-serum, sterilized at 68° C., pneumococci usually develop well-marked capsules. In some of the serum media, streptococcus cultures may at times have demonstrable capsules.

All streptococci examined, which by the usual methods would be classified as *streptococcus pyogenes*, have been found to possess cap-

sules. These were demonstrated by special methods and stains devised during this work.

These methods and stains are also especially applicable to the demonstration and staining of pneumococcus capsules.

In the light of the demonstration of capsules on streptococci, the usual morphological basis of differentiation of streptococci from pneumococci appears insecure.

This is also true of the separation of the organisms described as capsulated streptococci into species distinct from streptococcus pyogenes, or from true pneumococci.

This separation does not seem warrantable unless other and especially well-marked cultural differences are demonstrated, which distinguish such capsulated streptococci from pneumococci or from streptococcus pyogenes. Well-marked examples of organisms which would probably be described as capsulated streptococci have been examined during these experiments. They have been found to correspond to streptococcus pyogenes when cultivated in the media described in this paper.

It is a pleasure to acknowledge my indebtedness to Dr. Edward Leaming for the photographs reproduced in the plates.

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DESCRIPTION OF PHOTOGRAPHS.

PLATE XXIV.

Fig. 1. Pneumococcus I from Loeffler's blood serum. Spread in condensation water. Stained by potassium carbonate method. 2000 \times .

Fig. 2. Pneumococcus I from heart blood of rabbit. Copper sulphate method. Mounted in balsam. 2000 \times .

PLATE XXV.

Fig. 3. Pneumococcus II from serum agar. Spread on cover-glass in serum. Pot. carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 4. Pneumococcus I from sugar free broth. Spread on cover-glass in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 5. Streptococcus I from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 6. Streptococcus II from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 7. Streptococcus IV from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 8. Streptococcus VII from glycerine agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

PLATE XXVI.

Fig. 9. Streptococcus IX from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 10. Streptococcus XI from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 11. Streptococcus XIV from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 \times .

Fig. 12. Capsulated bacillus from agar. Spread in serum. Potassium carbonate method. Mounted in balsam. 1000 \times .

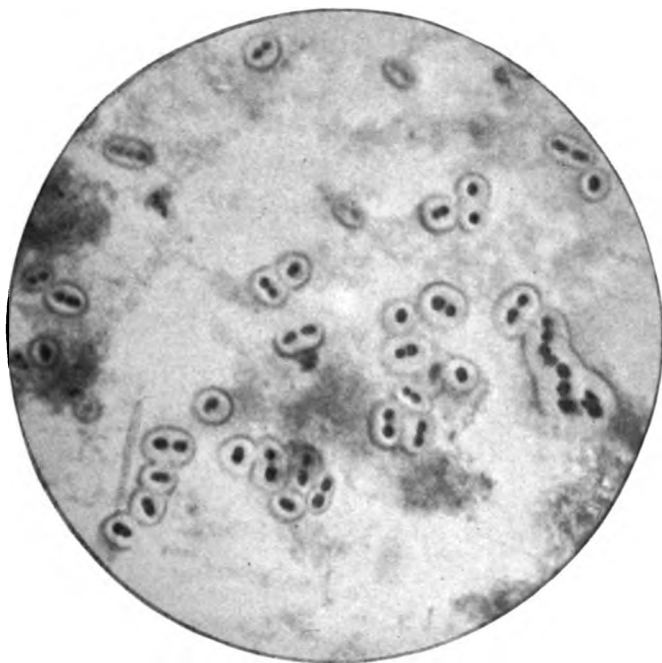


FIG. 1.

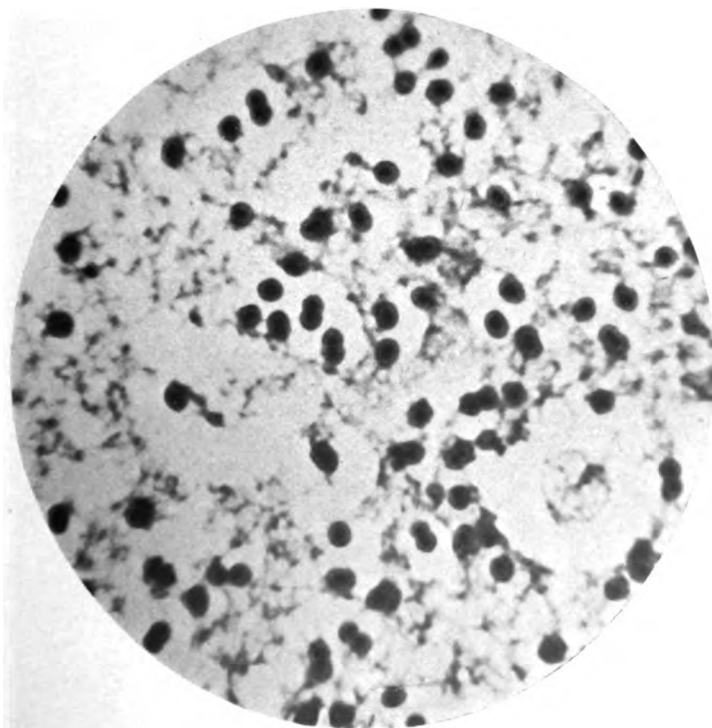


FIG. 2.



FIG. 3.



FIG. 4.



FIG. 5.



FIG. 6.



FIG. 7.



FIG. 8.

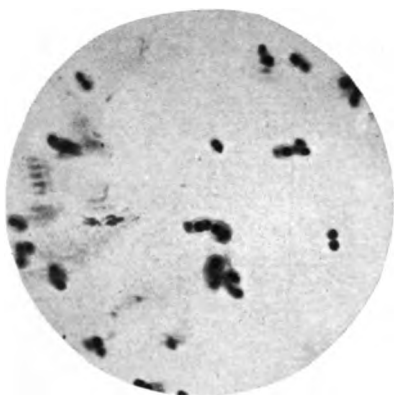


FIG. 9.



FIG. 10

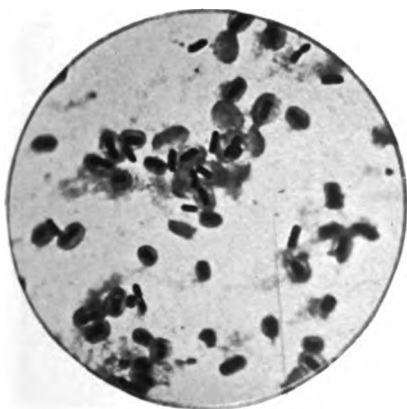


FIG. 11.

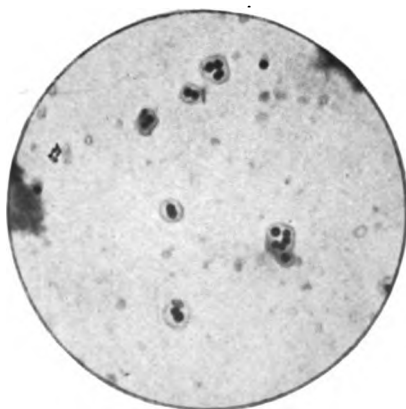


FIG. 12.

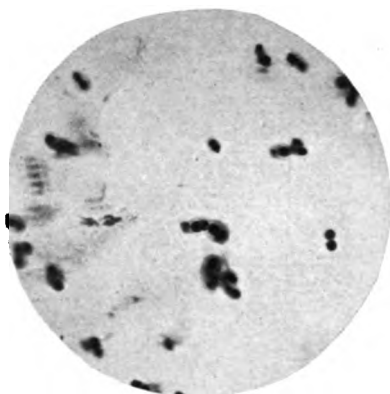


FIG. 9.



FIG. 10

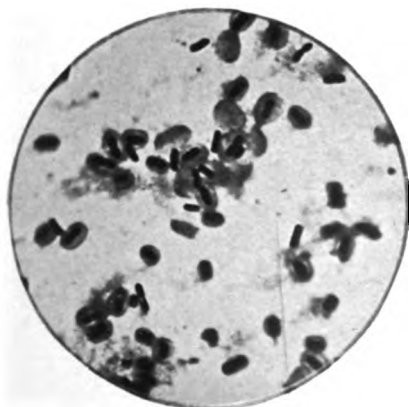


FIG. 11.

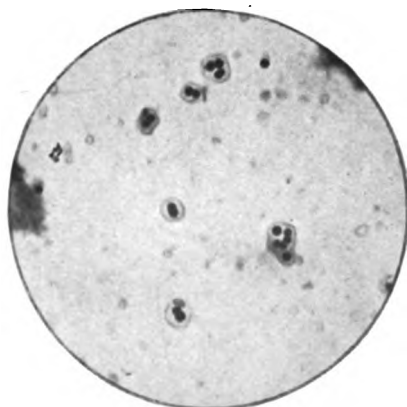


FIG. 12.

ON FERMENTATIVE AND AGGLUTINATIVE CHARACTERS OF
BACILLI OF THE "DYSENTERY GROUP." *

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I.

INTRODUCTORY. — The chief object of the present paper is the communication of the results of certain studies on the fermentations and agglutinations of the bacilli which are now generally considered etiological factors in acute dysentery and allied intestinal diseases. It has been thought well, however, at this time to preface these investigations with a critical review of the development of our knowledge of these organisms, and to examine the claims advanced in establishment of their etiological significance.

It has only been hand in hand with the development of some of the more recent methods of research that investigations into the etiology of dysentery and allied intestinal diseases have led to significant results. With these later investigations we are alone concerned in this review, as a study of the results obtained is instructive and serves as an illustration of the application of the more refined modern methods in the solution of a practical problem. A careful review of the subject emphasizes the true advances which may be made when the newer methods are intelligently and thoroughly applied to the study of disease and the identification of organisms. It makes apparent also the errors which may occur through an inadequate or inefficient application of these methods.

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HISTORICAL.

The Development of our Knowledge of Dysentery Bacilli and of their Geographical Distribution.

In 1898 Shiga¹ published the results of his investigations of the epidemic dysentery of Japan. He claimed to have found one and the same species of bacillus in the dejecta or the intestinal mucosa of thirty-six cases of dysentery, and that the bacilli showed marked agglutination when mixed with the serum of dysentery patients, and that other bacteria isolated from the dejecta or intestinal walls of the patients showed no reaction with their sera. Further, he states that the bacillus was not found in the dejections of patients suffering from other diseases nor in those of normal men, and that when tested against the blood or serum of such people the bacillus was not agglutinated.

Shiga's first paper was a brief communication, but we gather from this and his two subsequent papers² (1898-1901) that the bacillus was a short rod rounded at the ends, morphologically very similar to the typhoid bacillus and like it inclined to involution forms. The organisms generally occurred singly, more seldom in pairs. With methylene blue the rods were stained most intensely at the ends. Gram's method decolorized the bacillus. The organism was a facultative anaerobe. A very slow motility was noticed. This motility, according to his 1901 paper, was not so active as that of typhoid bacilli; little change of position was shown, and it was with difficulty distinguished from molecular movement.

The colonies on agar and gelatin were not especially characteristic, resembling those of the typhoid bacillus, although Shiga first considered that the gelatin colonies differed from the typhoid, not showing the typical grape leaf formation. This he subsequently attributed to differences in the composition of the gelatin. Gelatin and blood serum were not fluidified. Growth on potato resembled that of typhoid bacilli, being scarcely visible at first, but after about a week turning reddish-brown. Broth cultures showed a fairly intense clouding with moderate deposits after some days at 37° C. No pellicle was formed.

No gas formation took place in grape-sugar bouillon or agar. Milk was not coagulated. Litmus whey became slightly acid after one to three days at 37° C. After five to seven days it became alkaline again, and then of a deeper and deeper blue. Indol was not demonstrated in broth or in pepton water. Alkaline media were considered most favorable. The bacillus was to be differentiated from the typhoid organism only by its less active motility and by specific agglutination.

In the beginning of the disease the bacilli were difficult to find, but were often present in nearly pure culture in the mucous and bloody dejections during the second half of the first week. In the intestinal mucosa they were very plentiful, and nearly pure in fresh catarrhal or pseudo-membranous lesions and in the deeper layers of ulcerous processes. They were often present in the swollen mesenteric glands, but not in the liver or

spleen. They were constantly present in all cases of dysentery, and their appearance and disappearance corresponded to the stages and severity of the disease

Some of Shiga's serum tests showed an agglutination as high as 1:130, but generally 1:20-50; and at times they were negative in very light cases. Thirty-four tests of normal or other bloods were always less than 1:10. The agglutinating strength of the blood developed, in general, hand in hand with the disease process. In severe cases it was usually over 1:50, and in the average cases 1:20-50. In light cases it was scarcely to be made out or was absent. In lethal cases it was very weak, 1:10, 1:20.

The agglutinating power appeared first in the second or third week, reached the highest point during convalescence, and then gradually faded. At times it did not appear before the sixth week, and Shiga considered the Widal reaction in dysentery of little use in diagnosis.

As a point in the etiological chain, Shiga states that injections of dead dysentery bacilli caused much lighter inflammatory processes in dysentery convalescents than in normal people, and that Pfeiffer's reaction was especially marked during convalescence.

Shiga³ also claimed (1901) that immune serum (due to inoculations of this bacillus) had preventive and therapeutic action against dysentery.

In 1899 Flexner⁴ investigated cases of dysentery in the Philippines. The results of these investigations were made public in 1900, and the description of the bacillus isolated corresponds in all important points with that of Shiga's organisms. The bacillus was described as showing moderate motility and was Gram negative. Gelatin was not fluidified, and the colonies resembled those of *B. typhosus*, being more nearly like them when first isolated than after a period of cultivation outside of the body. The growth on potato after some days was a little elevated and of a pale brown tint. On unfavorable potatoes the growth was slight, moist, and membranous, resembling, except for the greater amount of moisture, that of *B. typhosus* when typical.

Sugars — glucose, lactose, and saccharose — were not fermented "gaseously." In glucose media a moderate acid production took place. Litmus milk assumed, after twenty-four to seventy-two hours, a faint lilac tinge. After the lapse of from six to eight days alkali began to be produced, which increased in amount until the litmus was rendered deep blue. No coagulation took place. Broth was clouded diffusely and a sediment formed. No pellicle was produced. Flexner noted the production of indol in some cultures, but says that even in sugar-free bouillon it may fail to appear or it may be produced in small quantities only. In this paper the serum reactions receive only a slight mention, Flexner stating that suitable cultures of this organism, when tested for the agglutination reaction with the blood serum of persons suffering from dysentery, gave in many cases a positive result.

The organism described by Flexner was abundant in the stools of acute

cases, in which it was at times the predominating organism. It became more difficult to find as the cases progressed towards recovery or chronicity. It could be cultivated from the dejecta during life, and the intestinal contents, mucous membrane, and mesenteric glands in fatal cases. The organism was not found in the evacuations of healthy subjects or of persons suffering from beri-beri.

Strong and Musgrave's* description (1900) of bacilli isolated from dysentery cases in the Philippines corresponds essentially to that of Flexner; but Strong was unable to demonstrate indol in cultures in pepton solution or in sugar-free bouillon. The virulence of the organism was said to vary greatly in different cultures. Strong reported that the disease had been produced in man on one occasion by ingestion of dysentery bacilli.

The blood serum of the acute and sub-acute cases was said to have usually a marked agglutinating action on this bacillus. The reaction was in some instances present as early as the third day, and was usually marked by the fifth or sixth.

Nearly simultaneously with the papers of Flexner and of Strong and Musgrave, Kruse* published his investigations (1900) of an epidemic of dysentery occurring in Germany. His observations were of the greatest importance, and his careful investigations and accurate descriptions largely formed the starting point of the further advances which have been made in the knowledge of the etiology of dysentery.

His organism was described as forming colonies and growth on gelatin and agar practically like those of *B. typhosus*. Like this bacillus, no gas was formed from grape sugar, and the growth in milk and on potato, and even in Piorkowski's urine gelatin, resembled that of *B. typhosus*. Parallel cultures on potato, however, would differentiate them. This differentiation, however, he considered unnecessary, since his organism was a plump, thick rod and *non-motile*, while typhoid bacilli are slender and motile. Kruse's animal experiments were negative. The organisms were regularly found in fresh cases of dysentery, even in pure culture. This finding in itself Kruse would not have considered of importance if the bacilli had not been well characterized or had been found in normal persons or in other diseases. He had, however, in ten years' work on intestinal bacteria never met with such an organism.

Among eighty-eight cultures isolated in Naples by Germano and Maurea, the study of which he followed, there was not one which did not ferment grape sugar with gas production; and among twenty-five cultures that he and Pasquale cultivated from cases in Egypt, only three gave no gas. He notes that the few bacilli of this character described in the literature are *motile*.

Kruse's serum tests are important, and definite figures are given. His cultures, when mixed with the blood serum of patients who had been ill over seven days, agglutinated regularly in a dilution of 1:50. Many of the twenty-five sera tested gave reactions in dilutions of 1:100, 1:250,

and one, 1:1,000. Twenty-five tests with normal sera were negative or positive only at 1:10-20, and exceptionally as high as 1:50. All other bacteria isolated and tested gave negative results in high concentrations of serum.

Kruse concluded in this paper that his organism must be different from Shiga's on account of Shiga's claim of motility and the difference in his description of colonies. This led to a discussion between Shiga and Flexner, who claimed motility for their organisms, and Kruse, who had never noted this in any of his cultures.

Curry,⁷ like Flexner and Strong, also reported on dysenteric diseases in the Philippines, and the finding, in several cases of acute dysentery which came to autopsy, of a bacillus apparently identical with that described by Shiga and Flexner. This culture he compared with a Shiga culture sent to him by Kitasato, and could determine no difference.

In June, 1901, Kruse⁸ contributed a second paper. His further work on serum reactions led him to change his mind about the 1:50 agglutination of his organism in normal serum, as he believed that he had been dealing with the serum of people who had previously had dysentery. He had compared cultures from the Philippines sent to him by Flexner, and one of these cultures, with the exception of a slightly less abundant growth by the Flexner bacillus on all media, agreed with his own cultures.

The character of the serum reaction of the Flexner organism was different from his, both in dysentery serum from patients and immune animals. Whereas his bacillus clumped in the usual manner, Flexner's bacillus gave Pfaundler's reaction in the same dilutions. The same differences, however, he had noted in some of his own cultures, and he concluded that Flexner's bacillus was closely related and simply a variety of his species. Although he had not examined Shiga's cultures, he believed from the similarity of description of Shiga's and Flexner's organisms, and the geographical position of Japan and the Philippines, that they were one and the same, and, consequently, that his own and Shiga's bacillus were very similar. This article is polemical, but Kruse very justly remarks that "in bacteriology we have no superabundance of characters that are of differential value," and he asks where it would lead us if we failed to lay stress on the presence or absence of motility, fermentation activities, and pigment formation, as some authors wish to do. At the same time he believed that we would probably, in the course of time and on the ground of true etiological criteria, yet learn to differentiate a series of dysentery organisms, just as we to-day recognize more than one species of cholera, diphtheria, and meat-poisoning organism.

The most important part of this paper (in the light of later developments) deals with a description of organisms coming from cases which he designated, unfortunately as it now seems, "pseudo-dysentery of insane asylums." He examined the stools of twelve insane patients suffering from a form of dysentery, and material from two autopsies. In these fourteen cases he was never able to isolate an organism corresponding in all characters to

his true dysentery bacillus. In the case of one patient and the two autopsies, however, he isolated organisms which he could not distinguish morphologically or culturally from this bacillus, but which differed from it in their serum reactions. These cultures agglutinated at 1:100 in the serum of the dysenteric insane patients, but his other organisms did not. In normal serum they often agglutinated at 1:50. This is higher than the agglutination of dysentery cultures. By careful comparison in the serum of patients and immune serum from animals, he not only showed that these organisms were different from his dysentery cultures, which, no matter what their source, were found to be alike, but that they showed differences among themselves even of a "specific nature," and apparently fell into two or more varieties or species. One of these organisms, culturally and by its serum reactions, showed itself practically identical with one of the cultures he had received from Flexner and designated "America." This is not the particular Flexner culture which Kruse considered resembled his own epidemic dysentery bacillus so closely.*

Kruse's conclusion that the dysenteric diseases of the insane have generally a different etiology from epidemic dysentery may have been premature, and in the light of our present knowledge seems incorrect; but the observation, nevertheless, that his cases were due to organisms different from that which he had constantly found in epidemic dysentery, and further that these organisms were of several varieties, one of which, at least, corresponded to one of Flexner's cultures, was correct, and as we shall see has been fully borne out by later researches.

Flexner* (1901, October) considered as premature Kruse's conclusions in regard to the difference of the etiology of the dysentery of the insane, and from a comparative study of morphological and cultural characteristics and serum reactions of various cultures — Shiga, Kruse, Strong, Porto Rico, and Flexner ("Gray" and "Harris") — came to the conclusion that the differences between them were slight and unimportant. Of the serum reactions he has the following to say: "The serum reactions have been of greatest importance. They are, moreover, unmistakable in significance; they indicate close relationship between the bacilli from Japan, Manila, Porto Rico, and Germany, and they further render probable the identity of the epidemic dysentery of this country (United States) with

*This bacillus "America," so far as I am aware, is one of Flexner's Philippine cultures, probably either "Gray" or "Harris." The other culture is in all probability the same culture which Professor Flexner sent to me early in 1901, and which, as later developments have shown, corresponds culturally to Kruse's organism, although, as we noticed from the first, it grows less abundantly than Kruse's cultures and is more slender, as noted by Kruse. This organism is of special interest, for we received it from Flexner before the American isolations were commenced, and it is supposedly, therefore, of Philippine origin. It is entirely different from the "Gray" and the "Harris" of Flexner, which are described by Europeans as the "Flexner" bacillus. This is of interest as indicating the isolation by Flexner of two different organisms in the Philippines, one corresponding closely to Kruse's bacillus of dysentery and the other to his bacillus from insane patients.

that of the East and Germany." No definite figures are given, but he remarks that certain strains were less active agglutinators and required a longer time or greater serum concentration before agglutinating. Pfaundler's reaction was also noted in some cases. The results of his comparative study, he concludes, "leave no doubt of the identity of the several bacilli with which I have worked. They indicate, moreover, that the acute dysenteries tending to appear in groups of cases and in epidemics, whether in the far East, Germany, or the West Indies, are due to the same organisms." It does not seem possible that Flexner determined accurately the terminal limits of the serum reactions of his various cultures in the different sera, otherwise he must certainly have been impressed by the wide differences in the terminal agglutination of some of these cultures.

Marckwald,¹⁰ in 1901, as a link in the chain of evidence of the etiological relation of Kruse's (or Shiga's) bacillus to dysentery, reported a case of epidemic dysentery in a fetus. The mother had dysentery and aborted at the sixth month. The child died in a few hours. It had lesions suggestive of the early stages of dysentery, and the bacilli were found in its intestinal contents and also, in small numbers, in the heart's blood.

Spronck¹¹ (1901) described an organism isolated by him in Utrecht, (Holland), from patients suffering with dysenteric symptoms, which clinically were not so typical as in acute epidemic dysentery. In autopsies, however, typical diphtheritic lesions of the colon were present, as in epidemic dysentery. In five out of seven of the cases examined the organism was present in nearly pure culture. This bacillus showed great similarity to the Shiga-Kruse organism of epidemic dysentery, but tested in the serum of a horse, which had been immunized against the bacillus of epidemic dysentery (agglutination titer, 1 : 1,000-1,500), the Utrecht bacillus showed practically no agglutination. Spronck says that apparently the bacillus belongs to the group of Kruse's pseudo-dysentery bacillus, but that he does not doubt its etiological significance since it was present in such colossal numbers in the stools, and was agglutinated strongly in the blood serum of the convalescents, while these sera did not agglutinate *B. dysenteriae epidemicæ*.

This communication is of importance and of special interest, since it is the first reported instance in which any investigator since Kruse had carried out serum tests of differential value, and recognized and associated the so-called "pseudo"-dysentery bacillus etiologically with dysentery approaching the acute epidemic form in type.

Vedder and Duval,¹² in February of 1902, published the results of their investigation carried on in the summer of 1901, under the direction of Professor Flexner, of various outbreaks of dysentery in the eastern United States, in Philadelphia and Lancaster, Pa., and in New Haven, Conn. Some of their cases occurred in institutions and among the insane. They made a comparison also of the original cultures of Shiga, Flexner ("Gray" and "Harris"), "Strong," and "Kruse." These they considered

indistinguishable from each other and from the organisms isolated by themselves, either culturally or by their serum reactions, although what they consider minor differences were shown, particularly in the serum reactions. They concluded that the acute dysentery of the United States is due to a bacillus indistinguishable from that obtained from the epidemics of dysentery in several other parts of the world; that the sporadic and institutional outbreaks of acute dysentery are caused by the same microörganism, and that this organism is identical with that causing epidemic dysentery; and that the cause of acute dysentery, whether sporadic, institutional, or epidemic, is *B. dysenteriae* Shiga.

Motility was not discovered in the bacilli obtained by them. They, however, claimed to have demonstrated flagella on the cultures Shiga, Kruse, Strong, and Flexner (see Flexner, 1901).

The value of this work is confined to the demonstration of the fact that organisms resembling Shiga's and Kruse's dysentery organisms culturally are to be found in dysenteric cases in the United States.

Their serum tests as *differential* studies are of little value, and tend in no way to disprove Kruse's observations. One culture, "Seward," widely known as "New Haven," and studied here and abroad, has since been shown to correspond absolutely to Kruse's cultures from epidemic cases. This in nowise excluded the possibility that other cultures isolated from other cases or epidemics did not correspond to those of the so-called pseudo type. In fact, a later study showed this to be the case.

Müller¹³ studied an epidemic of dysentery in Südsteiermark and reported, in 1902, the finding of a non-motile organism giving serum and cultural reactions similar to Kruse's bacillus. He also noted the isolation of *coli anaerogenes* (Lembke), a motile, non-gas-forming organism, in some of the cases.

Hiss,¹⁴ in 1902, reported that dysentery bacilli could be distinguished from typhoid and colon bacilli if studied in tubes of semi-solid gelatin agar, plus dextrose, at 37° C. It was determined by this method that none of the dysentery cultures studied — Flexner, Kruse — showed the slightest motility, while typhoid and colon bacilli, as was already well known, spread rapidly and clouded the medium partially or throughout, the colon also producing gas. This medium was thus shown to be of value in identifying dysentery bacilli from colonies in plates, as all motile or gas-producing organisms could be immediately excluded by observation of transfers from suspected colonies.*

* There is an obvious advantage in this over the use of glucose agar in which gas-producing organisms alone are excluded, while the motility has to be further studied in hanging drop, with the attending uncertainty of distinguishing motility from molecular movement. Since this report many dysentery organisms have been placed in this medium immediately from the isolating plates, and no motility, as judged by diffuse clouding, has ever been observed. This speaks strongly against the observations of Shiga, Flexner, and Strong as to the motility of cultures, although it is possible that the acid evolved by the organisms from the glucose may inhibit a slight motility. This effect has never been noted on truly motile organisms. The question of motility of these organisms is, however, still an open one.

Klopstock,¹⁵ in 1902, also contributed to the differentiation of dysentery bacilli from typhoid and colon bacilli. Basing his work on Barsiekow's use of nutrose as a base for fermentation studies, he proposed the use of a medium composed of nutrose, one per cent; NaCl, one-half per cent; glucose, one per cent, and lactose, one per cent, in distilled water. This was tinged with litmus solution and sterilized in tubes. Tests in this medium showed that dysentery bacilli, in twenty-four hours at 37° C., gave a visible acid reaction, but no gas or clouding; the typhoid bacillus acid formation and clouding; and the colon bacillus acid, coagulation of the casein, and also gas formation. Later the dysentery tubes clouded slowly, and the typhoid gave a precipitation of the casein.

In September, 1902, Duval and Basset¹⁶ made an important preliminary communication on the etiology of the summer diarrheas of infants. Their research was made at the Thomas Wilson Sanatorium, Baltimore County, Maryland. The dysenteric bacillus was present, they say, in forty-two cases of typical summer diarrhea, often in large numbers in the stools of the acute cases, but was secured with difficulty from cases of mild character and from those of long duration. They believed that the bacilli isolated from different cases of the disease were identical and agreed in morphology, cultural features, pathogenic properties, and reaction to specific serum, with the dysenteric bacillus isolated from cases of acute dysentery in adults by Shiga, Flexner, and Strong, Kruse, and Vedder and Duval. The bacillus was not found in the stools of twenty-five healthy children nor of those suffering with simple diarrhea, marasmus, or malnutrition; nor did the blood of these latter individuals agglutinate the dysenteric bacillus. They thought that their findings justified them in concluding that the summer diarrheas of infants are caused by *B. dysenteriae* of Shiga, and are, therefore, etiologically identical with acute bacillary dysentery of adults.*

Shiga,¹⁷ in his "Weitere Studien über den Dysenteriebacillus," October, 1902, compared the action of immune serum on Shiga and Kruse organisms, and found that both from the agglutinating and bactericidal stand-points its action is the same on both of these organisms. In comparing the action of these two organisms with Flexner's in this immune serum, he stated that he found a proagglutinoïd zone (a zone of non-agglutination) for the Shiga and Kruse bacilli in the more concentrated dilutions of the serum, whereas this is absent for the Flexner organism. He determined that when this serum was saturated with a Shiga culture all the agglutinins and proagglutinoids for Kruse's organisms were absorbed by the bacilli, and when these were removed by centrifuging, the serum no longer agglutinated fresh additions of Kruse or Shiga bacilli, while its limit for the Flexner bacillus was but little changed. When a fresh specimen of the serum was saturated with Flexner's bacilli, the agglutinins for these bacilli were removed; the agglutinins and proagglutinoids for the

* The full report has just appeared. See Studies from the Rockefeller Institute for Medical Research, ii, 1904.

Kruse bacillus were little affected. This is very important as showing a difference in the receptor apparatus of these organisms. Shiga stated that the serum used, however, had not been produced through immunization with his original cultures alone, but by several cultures which may have had slightly different receptor apparatuses, thus giving rise to different agglutinins. From these experiments Shiga concluded that his original culture and Kruse's culture, so far as receptor apparatus goes, are fully identical, while these two varieties possess with the Flexner culture identical, as well as different, receptors.

Shiga also claimed to have succeeded in so transforming a Kruse culture by ten passages through milk that its receptor apparatus became identical with that of the Flexner culture. This must not be taken too seriously until further confirmed, and Lentz, in fact, as stated later, denies that this can be done, and believes that the organisms are distinct in their receptor apparatus.

Park and Dunham,¹⁸ in October, 1902, described an organism as the Shiga bacillus, which they found in a small outbreak of dysentery occurring at Seal Harbor, Mt. Desert, Me.

An important point in its biology noted by them is contained in an added note, in which they say: "The bacilli from the Seal Harbor and New York cases agree in agglutination properties with those sent us by Dr. Flexner, obtained from Manila and Baltimore, but differ from those obtained by Shiga in Japan and Flexner in New Haven. They also differ from the latter in producing *indol* in pepton solution." We have seen, however, that this determination of indol production had first been made by Flexner in the case of some of his Philippine cultures, but was apparently not looked upon by him as of differential value.

Martini and Lentz,¹⁹ in November, 1902, published an article on the differentiation of dysentery bacilli by means of agglutination. They primarily tabulated the characters of a number of dysentery and "pseudo-dysentery" bacilli. Only a few of the organisms which they classify as "pseudo-dysentery" bacilli are, however, of interest, since many of those in their list were either actively motile or produced gas, and should at no time have been confused with any of the organisms described by Kruse or Shiga. The agglutination tests were made with the serum of a goat immunized with Shiga's culture. In this serum Kruse's bacillus, Duval's "New Haven" culture, and a number of the organisms isolated from epidemics in Germany and considered identical with Kruse's organism by their discoverers, were found to correspond to Shiga's organism. Two Philippine cultures of Flexner's (probably "Gray" and "Harris") and Strong's culture, as well as many so-called pseudo-dysentery cultures, including Kruse's pseudo-dysentery bacillus, having cultural characters, so far as known, similar to the organism looked upon as the etiological factor in dysentery, were found to be practically not affected by the serum, refusing to agglutinate in dilutions over 1:25-50. The serum of a rabbit immunized with one of Flexner's Philippine bacilli agglutinated the two

Flexner Philippine organisms at 1:4,000; but did not affect the "New Haven" and Shiga cultures above 1:25, or the culture of Strong above 1:50. This research is of interest because of the number of organisms compared and as supporting the work of Kruse and that of Spronck, which had already very clearly indicated a difference between the agglutinative characters of the Kruse organism and the so-called "pseudo-dysentery" type, in which Flexner's organisms are included. It is of further interest, as it indicated a marked difference between Flexner's Philippine cultures and the Philippine culture of Strong, the "Strong" organism refusing to agglutinate not only in Shiga immune serum, but also in the Flexner immune serum. Martini and Lentz called attention to the inadvisability of using the serum of dysentery convalescents in an endeavor to determine the identity of organisms from different dysentery patients, as many organisms distinctly different in agglutinations in highly immune serum will be found to show practically the same agglutinations in this serum.

Simultaneously with this article Lentz²⁰ published the results of comparative cultural research with dysentery and "pseudo-dysentery" bacilli. By comparing these organisms in media of the formula recommended by Drigalski for use in isolating typhoid bacilli, but to which in the place of lactose various sugars and the alcohol mannit were added, he was able to determine physiological differences between the organisms which correspond to the Shiga-Kruse bacilli in agglutination reaction, and those belonging to the Kruse "pseudo-dysentery" or Flexner type. The principal difference was that the Shiga-Kruse bacilli did not affect mannit, while the "pseudo-dysentery" bacilli, including Flexner's and Strong's Philippine cultures, fermented mannit, giving rise to a distinct acid reaction in the medium. The mannit medium was the only one serving to differentiate the true dysentery from all of the pseudo type. The Flexner organisms and others of the "pseudo-dysentery" bacilli, however, fermented maltose, while the Shiga-Kruse type left it at the end of forty-eight hours practically unchanged, as did also Strong's bacillus. Dextrin showed practically no differentiation in forty-eight hours. The Flexner, Strong, and "pseudo-dysentery Kruse" behaved in this as did the Shiga-Kruse organism. Dulcitol, Fructose (saccharose) and Inulin were said not to be of differential value.

In January, 1903, Hiss and Russell²¹ described a bacillus (bacillus "Y") from a case of fatal diarrhea in a child, which by ordinary cultural tests and the absence of motility was found to resemble the Shiga-Kruse and Flexner bacilli. Immediately upon its isolation it was found, however, to differ from the Kruse cultures by its ability to ferment mannit. This observation was made independently of Lentz's work, which at that time had not become known in America.

In the comparative experiments of Hiss and Russell on the fermentative abilities of various dysentery cultures, a serum water mixture (beef serum, one part, distilled water, two or three parts) was used, since it was practically sugar free. To this was added the sugars to be tested in the

proportion of one per cent. By such tests it was found that the Kruse culture, a culture of Flexner's from the Philippines (not "Gray" or "Harris," but probably the culture referred to by Kruse), and Duval's "New Haven" (Seward) culture, fermented dextrose in this medium with the production of a solid acid coagulum, but did not affect mannit, maltose, saccharose, or dextrin. The culture of Hiss and Russell, on the other hand, not only fermented dextrose, but also mannit, with the production of acid and clouding of the medium. Maltose, saccharose, and dextrin were not fermented. Typhoid bacilli were found to produce acid in the presence of not only dextrose and mannit, but also maltose and dextrin.

Hiss* shortly after this had the opportunity to study the cultures "Gray" and "Harris" of Flexner, and Strong's Philippine culture, as well as Duval's "Baltimore" culture. The "Gray" and "Harris" Philippine cultures and the culture "Baltimore" were found to ferment dextrose, mannit, maltose, saccharose, and dextrin, and were thus differentiated not only from the Shiga-Kruse cultures, but also from the mannit-fermenting type described by Hiss and Russell, and Hiss reached the conclusion that at least three groups of organisms had been isolated from dysentery or diarrheic cases; one represented by the Shiga-Kruse culture, fermenting monosaccharids only; one by the Hiss and Russell culture, fermenting not only monosaccharids, but also the alcohol mannit; and finally, a group represented by the Flexner culture, fermenting monosaccharids and mannit, as well as maltose, saccharose, and dextrin.

The organism isolated by Hiss and Russell was shown to differ entirely from the cultures "Shiga," "Kruse," and "New Haven," in the serum of an immunized rabbit. This serum had for their organism "Y" a titer of 1:500. The Shiga, Kruse, and New Haven cultures, as well as Flexner's culture, did not agglutinate in it at any dilution. In normal beef serum it was found to agglutinate as high as 1:320, whereas "Kruse," "Flexner," and "New Haven" did not give a reaction in dilution over 1:10-20.

Rosenthal** in February, 1903, reported the finding of bacilli identical with a Shiga culture sent him by Kitasato, in dysentery cases in Moscow. The organisms were non-motile, and flagella stainings were negative. No indol was formed and a pellicle was not formed on broth cultures; in Piorowski's, medium colonies with threads occurred. In the serum of thirty cases tested the bacilli were agglutinated at the tenth or twelfth day in a dilution of 1:40, 1:50, and the strength gradually rose in many patients to 1:400. By the fourth to fifth week the agglutinating power sank. In one case it remained 1:100 on the fifty-second day. Typhoid and colon bacilli were not agglutinated by these sera.

Among fourteen autopsies, in three cases the bacilli were found in the mesenteric glands, but never in the liver, spleen, or blood. One case, however, is noted as an undoubted dysentery septicemia. Control

* See footnote to article by Hiss and Russell, loc. cit.

researches on normal stools, ordinary diarrhea, typhoid and tuberculous stools were negative.

Park and Carey,²² in March, 1903, described an epidemic of dysentery occurring in the town of Tuckahoe, near New York City. Carey isolated a culture from a fatal case. This organism resembled the Shiga-Kruse bacilli in not fermenting mannit, but was said to give indol in pepton solution after five days. It corresponded in agglutination with the cultures "New Haven" and "Shiga" when tested in the serum of a goat immunized against the mannit-fermenting culture "Baltimore," *i.e.*, did not react at 1:50, whereas Flexner's Manila culture, "Baltimore," Park and Dunham's "Seal Harbor" culture, and some New York cultures, all fermenting mannit, agglutinated up to a 2,000 dilution in the "Baltimore" serum. The authors remark that it is a "striking fact" that all the varieties of dysentery bacilli obtained from dysentery cases, which produce indol in large amount and develop acid from mannit, "agglutinate alike," while those which produce no indol, or only a trace, and do not develop acid from mannit, also group themselves together in so far, at least, as not reacting to some sera which powerfully agglutinate the others.

In June, 1903, Lentz²⁴ published a criticism of Shiga's claim that by passage through milk he had been able to convert Kruse bacilli into the Flexner type. Experiments by himself showed that even after twenty-three such milk passages the organism remained unchanged either in agglutination or cultural reactions, and he concluded that Shiga must have contaminated his culture with some Flexner bacilli during his experiments, and that so far as his own experiments go the receptor apparatus of the Shiga-Kruse and the Flexner organisms, in all specific sense, is totally different.

Gay,²⁵ in a study of types of dysentery bacilli in relation to bacteriolysis and serum therapy, considered that the variations in properties of the bacilli are important and worthy of consideration; but that the real question at issue is not alone whether the two varieties of bacilli are distinct, but whether they produce one or more kinds of pathological conditions in man. He can see no reason in calling any bacillus which causes dysentery a "pseudo-dysentery bacillus." He repeated the observation of Lentz and of Hiss and Russell, and confirmed their results as to the action of the bacilli in mannit. As regards the bacteriolytic action of serum on the two types, he said that bacteriolysis *in vitro* takes place when correspondence exists between the type of bacilli and of immune serum, irrespective of the strain of bacilli, and fails to occur when the types are not in conformity. He believed he had proved, however, that bacteriolysis is not an index of the protective power *in vivo* of anti-dysenteric serum; but it also shows that the protective power is greater for an organism of the corresponding type than for one of the other group. Almost twice the quantity of serum is needed to protect against the heterologous organisms than against the homologous type.

Gay and Duval,²⁶ in July, 1903, reported that they had found the two

types of bacilli — Shiga-Kruse and Flexner — in the same cases of dysentery. This was said to be true of three cases carefully examined by them, and further a re-examination of cultures saved from the Lancaster and New Haven epidemics showed that both forms had existed in these epidemics. They claimed, however, that all the bacilli isolated by Duval and Bassett from children suffering with summer diarrhea were found to be of the mannit-fermenting type.

They considered these cases to be of considerable importance on account of their bearing upon the question of the etiology of bacillary dysentery. The evidence all points, they thought, to the disease as being caused by these organisms which present certain cultural and agglutinative variations. On the other hand, they thought that the distinction of the organisms into dysentery and "pseudo-dysentery" bacilli, as attempted by Kruse and Martini and Lentz, is manifestly artificial and unwarranted, and that no such separation should, with our present knowledge, be undertaken.

Gay and Duval further believed, and the opinion was earlier expressed by Gay, that by the use of a higher immune serum than that used by Martini and Lentz they had been able to show that the variation in "cross agglutinations" between the Shiga-Kruse and Flexner types is "purely a relative one." Much doubt is thrown upon the value of the data upon which this opinion was formed, since the immune serum they used was from a horse, and, as has been shown by Lentz and by Park, normal agglutinins present in horse serum often give high agglutinations with the various types of dysentery organisms, and unless the original titer of the serum is known fair conclusions cannot be drawn.

In July, 1903, Knox²⁷ working at the Thomas Wilson Sanatorium, reported the isolation at this institution of dysentery bacilli from a further series of cases of dysentery in children mostly under one year of age. All the babies exhibited some form of intestinal disorder, but the stools of many of the series were largely fecal, and in these cases the organisms were present in comparatively small numbers. The discharges of twenty-five babies ranging in age from seven weeks to two years, suffering from no intestinal disorder, were carefully examined, but no dysentery bacilli were found.

No attempt seems to have been made to determine to what class of dysentery bacilli these organisms belonged, and serum reactions which were made are therefore without value. This same remark applies with very few exceptions to serum agglutinations made in America, and in many instances to those made in Germany and elsewhere.

Martha Wollstein,²⁸ in May, 1903, reported at the New York Pathological Society the results of the examination of the stools of one hundred and fourteen babies suffering from infantile diarrhea. The cases all occurred in New York City. No case, the stools of which contained blood as well as mucus, failed to show the dysentery bacillus; but many of the negative cases contained large quantities of mucus and undigested food without

blood. The tendency, she believed, in cases of infection with dysentery bacilli is towards the classical picture of dysentery as regards the clinical symptoms. The dysentery bacillus was found in thirty-nine cases out of the one hundred and fourteen examined. The bacilli isolated, with one exception, corresponded in cultural characters on the usual media to the Shiga-Kruse organism, but like the Flexner organism and the organisms of "pseudo-dysentery" fermented mannit and gave rise to indol. In one case, one of the most severe, the organism was of the Shiga-Kruse type, and did not ferment mannit or produce indol. All the mannit-fermenting organisms were said also to ferment maltose. Two of the cultures formed a thin pellicle on broth comparable to that described by Hiss in the case of his *Bacillus* "Y." All the bacilli reacted with a "Harris" (Flexner) immune horse serum in dilutions up to three thousand and with a "Shiga" horse serum to one to two hundred only. The culture which did not ferment mannit reacted with the "Shiga" serum in a dilution of one to three thousand, as did the Shiga and New Haven cultures. With the "Harris" serum it reacted only up to one to five hundred, as did also the Shiga and New Haven cultures. Reactions with the blood of the children were carefully tested with one of the isolated cultures and with the cultures "Harris" (Flexner) and "Shiga." No reaction was obtained in any case with the "Shiga" culture.

These serum reactions are the most valuable so far reported in America in a series of cases, since the character of the infecting organism was carefully determined in all cases and the agglutination tests were carried on systematically with the two best-known types of bacilli described in connection with dysentery.

Wollstein concluded that the serum reaction is uncertain during the first week, frequently present after the sixth day, but may be absent for two weeks and cannot be relied upon for early diagnosis in infants and young children.

Vaillard and Dopfer²⁰ reported in 1903 that they found the Shiga bacillus in an epidemic occurring at Vincennes. As they claimed that the organism isolated showed practically the same agglutination reaction as Shiga's, Kruse's, Flexner's, Pfuhl's, and Chantemesse's cultures in the serum from patients, as well as in that of an immunized goat, we are left in doubt as to the true agglutinating characteristics of the organism found, and must look with suspicion upon the results of their tests. It is at least impossible from their work to determine to which type the organisms conform.

Doerr²⁰ examined bacteriologically twenty cases of dysentery in Austria (Brück and Vienna) and was able to isolate from eight of them an organism corresponding culturally (non-mannit fermenter) and in its agglutinations to the Shiga-Kruse bacillus. The epidemic in Brück was of light character—among the one hundred and eighteen cases only one death occurred, and among the Vienna cases no deaths.

Agglutination tests with serum from convalescents and patients were positive. The titer was with one exception never under 1:50, and, as a

rule, as high as 1:200; twice in dilutions of 1:600. The action on Müller's and Kruse's cultures was the same as on his own.

Doerr used for his mannit tests a modification of Barsiekow's nutrose medium composed of mannit, one gram, NaCl, 0.5 gram, nutrose, one gram, and aq. dest., one hundred cubic centimeters, to the filtrate of which was added three cubic centimeters of Kaulbaum's litmus solution. In this medium the pseudo-dysentery bacilli (Flexner) gave an acid reaction.

Hetsch³¹ tested many cultures of Shiga-Kruse and the "pseudo-dysentery" bacilli in nutrose media. This contained one per cent nutrose, 0.5 per cent NaCl, and five per cent of Kaulbaum's litmus solution, and two per cent of mannit, or maltose, 2.5 per cent.

His tables show that with two exceptions all the so-called "pseudo-dysentery" bacilli tested ferment mannit, and give rise, as a rule, to a coagulation of the medium, and are thus differentiated from the Shiga-Kruse type (determined by agglutination). The two "pseudo-dysentery" cultures not fermenting the mannit in twenty-four to forty-eight hours are so manifestly not of the dysentery group that the mannit differentiation is without value. The maltose medium he did not consider so valuable as the mannit, as many of the "pseudo-dysentery" bacilli did not affect it more in twenty-four to forty-eight hours than did the dysentery bacilli, which all caused a faint reddening of the medium. If one studies the tables, one finds that the two Philippine cultures of Flexner and other similar cultures redden the maltose medium and cause a coagulation inside of forty-eight hours, while "Strong" and some other of the "pseudo-dysentery" stems react, as do the dysentery bacilli of Shiga and Kruse. The value of this distinction, pointed out by Hiss some time before as a means of separating varieties of the mannit-fermenting class, has been entirely overlooked by Hetsch, who with many of the Germans holds the mannit-fermenting organisms in slight esteem from the etiological standpoint, and simply seek to differentiate them from what they consider the true dysentery bacillus.

In an outbreak of dysentery in West Prussia, Jürgens³² investigated twenty-six cases, the clinical picture of which was like in all points to epidemic dysentery. From the dejecta of eighteen patients a bacillus was cultivated that resembled morphologically and culturally the Kruse dysentery bacillus, but was differentiated from this by its action in a highly immune animal serum. It seemed to be identical with or very closely related to the Philippine bacillus of Flexner. Like the Flexner bacillus, it produced acid in mannit agar. It was not agglutinated in marked degree by the serum of an animal immunized against Kruse's bacillus, but a Flexner immune serum agglutinated it in high dilutions. Likewise the serum of a rabbit immunized against the bacillus isolated, agglutinated the Flexner bacillus, but not Kruse's.

Jürgens' bacillus agglutinated in the serum of nearly all the patients and convalescents, while control tests with the serum of other patients failed.

For the first few days the agglutinins were not marked, agglutination even failing at 1:10; but later agglutinations in dilution of 1:100 to 1:500 appeared. The Kruse bacillus was not agglutinated. Jürgens argues for the etiological significance of this organism and against the etiological unity of dysentery. These cases and the isolated organism have been carefully and intelligently studied, and the results, like those of Spronck, form valuable contributions to the etiology of dysentery.

In December, 1903, Pillsbury³³ published the results of his investigations on the degree to which dysentery bacilli are agglutinated in the blood of non-infected persons, comparing the Shiga-Kruse and mannit-fermenting types in all sera tested. He concluded, as have most observers, that an agglutination at 1:20 is not rare, but further that this agglutination does not occur in the blood of young infants (under one year), rarely being present even in a 1:10 dilution. His conclusion, however, that the Shiga type of organism agglutinates more readily in normal and non-dysenteric bloods than do the mannit-fermenting types is contrary to the experience of other observers.

Firth³⁴ studied a series of dysentery bacilli including many well-known cultures, and communicated his results in December, 1903. The studies included fermentation and agglutination tests, but his observations on some of the well-known organisms are so at variance with those of other observers and my own knowledge of these organisms that one is forced to the conclusion that the cultures have by accident been miscalled in his paper, or that his observations have not extended over a sufficient time to make them reliable.

Hastings,³⁵ in Boston, studied thirty-five cases of dysentery in children. Of the twenty-eight cases from which organisms were isolated, twenty-three were said to be infected with the mannit-fermenting type of organism and three with the true Shiga type; and from two cases he claimed to have recovered both types, as did Gay and Duval in some of their cases. The three cases infected with the Shiga organism alone presented no marked difference from the rest of the cases.

Park, Collins, and Goodwin³⁶ have recently published a paper dealing with the dysentery group of bacilli and the varieties which should be included in it. The results of agglutination and absorption tests made by them indicate that the bacilli, isolated by themselves and others, fall into at least three types or varieties. These types or varieties display differences in their fermentative, as well as agglutinative characters, and the grouping thus arrived at substantiates the classification, based on fermentation tests, which was made by Hiss in 1903 (Medical News, February, 1903). The results of their agglutination tests correspond closely to those which we have obtained, and which will be found in another section of the present paper.

CRITICAL SUMMARY AND CONCLUSIONS DRAWN FROM THE
HISTORICAL DATA.

Briefly, then, from this review, in which I have endeavored to emphasize important points, we gather that in many countries scattered widely over the world, certain more or less closely allied bacteria not known to be normal inhabitants of any part of the gastro-intestinal tract have been isolated from the dejecta of persons exhibiting dysenteric and diarrheic symptoms, and from the intestinal contents, mucosa, and at times from the mesenteric glands of those dying of these diseases. The organisms, in severe cases with bloody and mucous discharges, are often found in nearly pure culture in the discharges, are more difficult to discover in the very early stages than at the height of the disease, and gradually disappear as the symptoms abate and the stools become again normal. Furthermore, although the intestinal discharges from patients suffering from other diseases, and those of normal persons, have frequently been investigated, organisms corresponding to these in type have not as yet been found,* nor does the literature contain records of organisms corresponding to them in all morphological, cultural, and biological characters.

This constant presence of the organisms in the dejecta and lesions of dysenteric patients, and their absence from the stools of normal people or those suffering from other diseases, would, a few years ago, have furnished, in the absence of definite experimental lesions in animals, practically the sole basis for the establishment of etiological relationship. Furthermore, the determination of the identity of organisms from different cases and epidemics would have depended almost entirely upon a correspondence of their morphological and cultural characters and of their pathogenic properties and toxic products, as determined by animal experimentation, criteria which we have long recognized as eminently unreliable. Prior, however, to Shiga's work on dysentery we had

* Several instances have been reported of the finding of "dysentery bacilli" in stools of normal children, but their presence in such persons, in places where dysentery is epidemic or endemic, does not prove that they are inhabitants of the normal tract.

begun to avail ourselves, in the elucidation of these questions, of our knowledge, then but recently acquired, that certain characteristic reactions take place with fair uniformity when the blood or serum of persons or animals suffering from an infection or artificial immunization is brought into contact with the true inciting organism or one of its species. In other words, we were at that time fully cognizant that such reactions, particularly agglutination, would in all probability take place when the blood or serum of dysentery patients was brought into contact with the true etiological bacterium, and, on the other hand, we also knew that correspondence of agglutination reactions in highly immune serum was the best guide, other things being equal, to the identity of organisms. We also recognized the equally important fact that the blood or serum of normal man or animals may cause similar reactions when bacteria are brought into contact with it, and that the mere occurrence of agglutination, therefore, irrespective of certain limits of dilution of the serum, is not of diagnostic or etiological importance.

Reference has been made to these well-known points, upon a due appreciation of which so much has depended in the attempted solution of the problem of the etiology of dysentery, since it must be obvious to one reviewing the subject as we have done that some of the confusion that has resulted in the interpretation of agglutination tests, and in the identification of the organisms isolated, has been due largely to a failure to comprehend the full meaning of these facts and procedures or to apply them scientifically and in extenso. The results, in some instances, have been rendered worthless either through the failure to recognize the variety of organism involved or to control agglutination results by comparative tests with more than one type of organism, especially after these types had been recognized as possible factors in dysentery.

A reference to the abstracts of the literature, however, shows us that where these tests have been carefully and intelligently used, strong evidence has been obtained of the specificity of the reaction between the organisms isolated

from the various cases and epidemics and the blood or serum of the patients. This is true not only of those cases and epidemics proved to be accompanied by the organisms so accurately described by Kruse, but also in the epidemics such as those described by Spronck and Jürgens, and the cases investigated by Wollstein, from which organisms corresponding to those called by Kruse "pseudo-dysentery" have been isolated, and the sera shown to agglutinate the isolated bacilli in high dilutions, but not the Shiga-Kruse organism.

We must conclude, then, from the results of these researches and agglutination tests that both the true Shiga-Kruse organism and the organisms which are morphologically and culturally closely similar, but ferment mannit and produce indol, have apparently an equal claim, so far as their presence in the dejecta of dysentery patients and the specificity of their serum reactions are concerned, to be looked upon as etiological factors in dysentery. Either this is the case or both must be equally rejected, since in the carefully investigated cases in which mannit-fermenting organisms have been isolated the serum of the patients has been found not to agglutinate cultures of Kruse's organism, and the cases cannot therefore be considered as due to it.

With the exception of the few instances in which animals infected with bacilli of either of these two types have responded with dysenteric lesions, and the cases in which people have accidentally or by design, as in Strong's Philippine prisoner case, ingested cultures of dysentery bacilli and this ingestion has been followed by dysenteric symptoms, the present claim of specificity of organisms occurring in dysentery cases and epidemics rests solely upon the constancy of their occurrence among dysentery patients, their absence in other cases, and the high degree of agglutinating power exhibited by the blood of dysentery patients towards these organisms of either type according to the epidemic.

The significance of the simultaneous occurrence of both types in the same case or epidemic cannot as yet be determined, as the reported cases are too few to base conclusions upon. In such epidemics and cases, it seems, however,

that the serum of patients exhibits an agglutinating power for both classes of organism. As this is not the case in other epidemics studied, the conclusion that the true Shiga-Kruse is always present or that the organisms are interchangeable forms is certainly not warranted.

In regard to the organisms themselves we have seen that Shiga recognized one type only, and that Flexner and Strong practically identified their organisms with that of Shiga, Flexner noting, however, that indol was formed by certain strains, but apparently placing no differential value upon this character. Their comparative serum tests, principally made with the serum of patients and convalescents even in comparing organisms from different localities, failed to indicate to them differences in the organisms studied.

Kruse's studies, on the other hand, led him to the recognition of several types of organisms, identical morphologically and culturally so far as he could determine, but differing materially in their serum reactions. One of these types isolated from cases constituting an epidemic of acute dysentery he looked upon as the etiological factor in this disease. The other types were isolated from insane patients suffering from dysenteric symptoms, and were designated by him as the bacilli of "pseudo-dysentery of the insane." These latter organisms were distinct in their serum reactions, and differed also from each other even to a "specific" extent in these reactions. He did not consider that these organisms were causative agents in acute epidemic dysentery, but looked upon them as possible factors in the dysentery of the insane. Kruse's investigations and descriptions of morphological and biological characters and agglutination reactions were so painstaking and accurate that, so far as fundamental facts go, little has been added to the knowledge of this group of organisms since, although some culture media have been devised which bring out physiological differences and fully substantiate the facts first made known by him.

That Kruse should at first have considered his organism different from Shiga's is not surprising, as he supposed the gelatin colonies to be different and that Shiga's bacillus was

motile. These differences were shown later not to exist, and the *practical* question of motility or non-motility must, I think, be considered to be overwhelmingly settled in favor of Kruse's determination of non-motility.

Although it is now certain that Shiga's culture, which has been widely distributed among bacteriologists throughout the world, corresponds in all essential points to Kruse's cultures from epidemic cases, he may, nevertheless, have isolated bacilli corresponding to Kruse's cultures from insane patients and not have recognized them by his method of study. This is not only not improbable, but is rendered fairly certain by statements already called attention to in regard to the agglutinins in his polyvalent immune serum.

Kruse's study of Flexner's organisms from the Philippines brought him to the conclusion that one of these organisms should be considered as practically identical with his dysentery cultures, but that another of them corresponded to his "pseudo-dysentery" cultures. This called forth a protest from Flexner, who studied various cultures — Shiga, Kruse, Strong, Porto Rico, "Gray," and "Harris" — and could determine no marked cultural differences and only very minor differences in agglutination. He could, in other words, determine no specific differences among the cultures. Vedder and Duval, as we have seen, failed also to determine specific differences by their cultural or agglutination tests, and were firmly of the opinion that all acute dysenteries, sporadic, institutional, and epidemic, were due to one and the same species. Spronck, on the other hand, immediately recognized the biological differences which Kruse had determined in organisms isolated by him from cases in Holland, but concluded that these organisms, although corresponding to Kruse's pseudo-dysentery type, were, nevertheless, etiologically connected with the epidemic.

The marked difference between the Shiga-Kruse organism of acute dysentery and the organisms simulating and for the most part mistaken for them was not fully recognized, especially in America, until the appearance of the results of Martini and Lentz's comparison of a series of dysentery and

dysentery-like organisms in immune sera, and of the studies of Lentz and of Hiss and Russell on the fermentation reaction of these organisms. From this time on fermentation tests were made, particularly in mannit, and differences in agglutination were more carefully considered in attempting to identify organisms derived from cases of dysentery. This has led to the demonstration of the fact that the mannit-fermenting bacilli are of equal, if not greater, frequency in dysenteric cases and epidemics, especially among children, than the non-mannit-fermenting or Shiga-Kruse bacilli, and that they apparently have an equal claim with the Shiga-Kruse bacilli, according to all tests, to be looked upon as the etiological factor in certain epidemics of dysentery as well as in institutional and sporadic cases.

II.

PERSONAL INVESTIGATION.—The observation first made by Kruse in his study of the bacilli of what he was pleased to call "pseudo-dysentery" cases that differences of specific value existed among the bacilli, and which was confirmed and made more apparent by the fermentation studies of Hiss, have failed, it is believed, to receive the attention they may merit, and it is the chief object of this paper to record the results of further experimental studies on this subject, which were begun in 1902, and which, with some intermissions, have been carried on up to the present time.

These studies deal with experiments on the fermentative activities of dysentery organisms from various sources, and with the serum reactions obtained with them in normal animal sera and in immune animal sera of high activity.

CULTURES TESTED. — The following organisms were employed in most of the tests: cultures of Shiga's, Kruse's, Strong's, and Flexner's bacilli sent me by Dr. Flexner. The Flexner cultures are those known as "Gray" and "Harris," and also the culture mentioned in the text as probably one of those studied by Kruse and found to correspond to his

own dysentery organism, and to which I shall, for the sake of brevity, refer as "Flexner." * Vedder and Duval's culture "New Haven" and Duval's culture "Baltimore;" a culture sent to me by Dr. Wollstein and known as "Wollstein;" the culture "Seal Harbor," isolated by Dr. Park; the culture usually known as "Y," isolated by Dr. Russell and myself, and two cultures isolated by myself from the intestinal mucosa of children who had died from dysentery; the cultures are known as "Diamond" and "Ferra." These three cultures were from New York cases. All the cultures mentioned, with the exception of "Diamond" and "Ferra," are well known generally, and have been extensively studied by other observers. Some of these cultures have been in my possession for over two years, all of them for over one year. The cultures show practically identical morphological and staining characters. Slight differences may exist in their morphology, but they cannot be considered of diagnostic value. None of the bacilli, so far as my own observations go, are motile, nor have they been so since coming into my possession. My own cultures have never shown the slightest indication of motility. None of them fluidify gelatin, and the variation in their growth characters on gelatin and agar are insignificant, likewise the differences on potato. Indol is formed in pepton solution by some of them — most intensely by "Gray," "Harris," "Baltimore," and "Wollstein;" less so by "Y," "Seal Harbor," "Diamond," and "Ferra."

"Kruse," "Shiga," and "New Haven" apparently do not form indol, or only the faintest trace.

None of them ferment any sugar with the production of gas. Fermentations leading to acid production are detailed below.

FERMENTATION STUDIES. — These studies were undertaken in the hope of accurately determining some of the physiological activities of the organisms, and thus to arrive

* There is every reason to believe, as stated before, that this bacillus is of Philippine origin. Dr. Flexner can give no information to the contrary, and it has been in my possession since early in 1901.

if possible at a definite classification. The value of rapid methods * of differentiation was kept in mind, even if these did not express truly fundamental differences, but the determination of physiological similarities or differences was our ultimate object.

All the organisms experimented with, as above noted, have been cultivated for many generations on artificial media—successive transfers on agar. In many instances the first fermentation tests were made immediately upon isolation; in other instances this was impossible, as the organisms were sent to me some time subsequent to isolation. Multiple tests have been made, and the final tests in all over a year after isolation. Whenever functions unsuspected from earlier or shorter tests became apparent, platings were made to determine the purity of the culture, and new studies were made from the various colonies; hence, the results here recorded are controlled.

GENERAL REMARKS ON THE MEDIA USED IN THE FERMENTATION STUDIES.—The fermentable substances tested were dextrose, maltose, saccharose, dextrin, and mannit. In some of the earlier experiments made in 1902, galactose, lactose, levulose, and inulin were also tested. In later tests these were omitted, since in the case of the monosaccharids—galactose and levulose—no differences of value were shown from those obtained with dextrose. In the case of inulin, tests showed that none of the organisms fermented it with acid production; lactose fermentations were always determined in the culture test in milk. In earlier experiments various glucosids were tested, but the results were either negative or unimportant, and their use was discontinued.

* In the opinion of the writer the early introduction of so-called "diagnostic" and short-cut methods, in the identification of organisms so little known as those comprising the group with which we are working, is to be deprecated, as they are apt to furnish not only in fermentation, but in agglutination tests also, incorrect or incomplete data and lead thus to erroneous conclusions. Much of the work already done on the etiology of dysentery has failed to prove as valuable as might have been hoped for from so large an expenditure of effort and time, owing to the failure to apply thorough agglutination tests, and to determine in extenso the fermentative functions of the organisms dealt with.

The monosaccharid — dextrose, the disaccharids — maltose and saccharose, the polysaccharid — dextrin, and the alcohol mannit were early found to be of value, and attention was therefore centered in them, and the nutrient bases by which they were accompanied, rather than on a larger and less readily handled series of fermentable substances.

The nutrient material and salts accompanying the carbohydrates and serving as the essential nutrient bases were varied in character and amount in the different experiments to determine their effect on the fermentative functions of the organisms. The exact composition and essentials of preparation will be given with the individual experiments. Fluid media only were used. The litmus used in all experiments, as indicated, is Merck's highly purified, made up in a five per cent solution. All observations were made at 37° C.

EXPERIMENTS WITH SERUM WATER MEDIA. *

This medium is composed of beef serum, one part, and distilled water, three parts, litmus one per cent. This can be boiled without coagulating, and is always boiled to kill enzymes previous to the addition of the sugars. Sugars are added in one per cent proportion, and the media tubed and sterilized on three consecutive days from ten to fifteen minutes. Color is a marked blue. When acid is produced in sufficient amount the medium is coagulated solid, a less amount turns it purple or red.

Dextrose. — All the cultures produce acid in dextrose and generally bring about a reddening and solid coagulation of the medium in twenty-four hours; very exceptionally the coagulation may be delayed a few hours longer. Typhoid bacilli give a solid acid coagulum in twenty-four hours.

Maltose. — Every culture tested will in time give rise to some acid production, indicated by a reddening or coagulation of the medium. This is distinctly not due to an impurity of the maltose. In some cultures it is not manifest for many days, but appears none the less surely. The rapidity with which maltose is fermented, however, is markedly different, and divides the cultures into distinct groups.

The cultures "Shiga," "Kruse," "New Haven," "Strong," "Y," "Seal Harbor," "Diamond," practically show no change for about thirteen to twenty-five days, and a solid coagulation only after twenty-five to thirty-five days. "Ferra" may give rise to distinct acid by the fourth, and solid coagulation by the fifth or sixth day (this organism is of special interest). "Baltimore," "Harris," "Gray," and "Wollstein" ferment

* Hiss. Science, 1902, March 7, p. 367; Ctbl. f. Bakt., 1902, xxxi, p. 302. Also Hiss and Russell. The Medical News, 1903, February 14.

maltose with ease, the serum being usually solidly coagulated in twenty-four to thirty-six hours. Typhoid bacilli coagulate the medium in twenty-four hours. "Strong" is the least active fermenter of maltose with which I have experimented. Tests, therefore, extending over only twenty-four to seventy-two hours would divide the organisms sharply as follows:

"Shiga," "Kruse," "New Haven," "Y," "Seal Harbor," "Diamond," "Ferra," and "Strong," no fermentation; "Baltimore," "Harris," "Gray," and "Wollstein," fermentation.

Saccharose. — "Shiga," "Kruse," "New Haven," do not ferment saccharose in the medium. The original stock culture of "Y," "Seal Harbor," and "Diamond" do not ferment saccharose in this medium in forty-five days. "Ferra" ferments saccharose in serum water, a slight acid change being apparent often after two or three days. The solid coagulation may be many days delayed. "Strong," "Baltimore," "Gray," "Harris," and "Wollstein" ferment saccharose in serum water, an acid reaction often being apparent in twenty-four hours, but coagulation may not take place even in forty-five days.

Here we have the organism separated into groups not precisely similar to those in maltose.

"Shiga," "Kruse," "New Haven," "Y," "Seal Harbor," "Diamond," not fermenting. "Ferra," "Strong," "Baltimore," "Gray," "Harris," and "Wollstein" giving acid reactions. Typhoid bacilli do not ferment saccharose in the medium and serve as a control of its purity,

Dextrin. — In pure dextrin we have a valuable aid to the differential diagnosis of dysentery organisms. In the serum water we find only organisms of the "Baltimore," "Harris," "Gray," and "Wollstein" type fermenting it. The fermentation as a rule is rapid, and the coagulation usually takes place in from one to three days. The culture "Gray," however, sometimes ferments dextrin slowly, and has to be observed over a number of days. "Ferra," as noted above, is an erratic organism, and at times shows some fermentation of dextrin, the acid production, however, usually occurs only after many days. So that we can group practically as follows:

"Shiga"	} No fermentation or very slight ("Ferra").	"Baltimore"	} Fermentation (1 to 5 days).
"Kruse"		"Harris"	
"New Haven"		"Gray"	
"Y"		"Wollstein"	
"Seal Harbor"			
"Diamond"			
"Ferra"			
"Strong"			

Mannit. — In the alcohol mannit, as is so well known, organisms of the Shiga-Kruse type are readily differentiated from the others. Our tests show a solid red coagulum in the case of all cultures, usually in twenty-four hours, with the exception of "Shiga," "Kruse," and "New Haven."

Thus :

" Shiga "	} No fermentation.	" Y "	} Fermentation.
" Kruse "		" Seal Harbor "	
" New Haven "		" Diamond "	
		" Ferra "	
		" Strong "	
		" Baltimore "	
		" Harris "	
		" Gray "	
		" Wollstein "	

EXPERIMENTS WITH ONE PER CENT PEPTON WATER.

This medium is composed simply of one per cent of Witte's pepton in distilled water, to which one per cent of the litmus solution is added. The sugars are in the proportion of one per cent. The medium is an intense blue. When acid is formed in this medium in sufficient amount, a slight precipitate is formed. The fermentations are, as a rule, similar to those in the serum water media.

Dextrose. — All cultures form acid, and the medium is red and shows the slight precipitate in twenty-four hours. Typhoid bacilli act in the same manner.

Maltose. — All cultures ferment maltose. The cultures " Baltimore," " Harris," " Gray," and " Wollstein " usually in twenty-four hours give the acid reaction and precipitate. The Shiga-Kruse type showed slight acid in fifteen days, complete in twenty-seven days. In the Hiss " Y " type the fermentation is long delayed. " Ferra " and " Strong " showed no acid production until twenty-five days, so that practically we may again separate " Shiga," " Kruse," " New Haven," " Y," " Seal Harbor," " Diamond," " Ferra," and " Strong " from the cultures " Harris," " Gray," and " Wollstein " type which ferment maltose rapidly.

Saccharose. — The Shiga-Kruse type showed no acid production. " Y " did not give rise to acid in this medium. " Diamond " gave some acid after thirty-five days, and " Ferra " after twenty-three days. " Strong " gave acid after twelve days. The " Baltimore," " Harris," " Gray," and " Wollstein " cultures formed acid more rapidly, the reaction usually being marked in nine to twelve days, and in this medium, as in serum water, these last organisms with the organism " Strong " are separated from the others by the more rapid and regular fermentation of saccharose.

Dextrin. — In this medium we have the following :

" Shiga "	} No fermentation.	" Baltimore "	} Fermentation.
" Kruse "		" Harris "	
" New Haven "		" Gray "	
" Y "		" Wollstein "	
" Seal Harbor "			
" Diamond "			
" Ferra "			
" Strong "			

The acid is produced in the "Baltimore" type often in twenty-four hours; at times, especially with "Gray," it is delayed.

Mannit. — "Shiga" "Kruse," "New Haven," no acid production. All other cultures acid and slight precipitate in twenty-four hours.

EXPERIMENTS WITH THREE PER CENT PEPTON WATER.

Similar to the preceding medium with three per cent pepton. These experiments need not be given in detail; they correspond, with one or two exceptions, to the tests of one per cent pepton. "Y" and "Diamond" gave an acid reaction in maltose after nineteen days; "Ferra" after seven days. "Baltimore," "Harris," "Gray," and "Wollstein" tubes were acid in twenty-four hours.

In saccharose "Y" gave acid after thirty-five days, "Ferra" after twenty-three, and "Strong" in twelve days. The "Baltimore" or "Flexner" type in nine to eleven days.

In dextrin only the "Baltimore" and "Flexner" type gave rise to acid, usually in twenty-four hours; "Gray" not as rapidly as the rest.

Mannit was not fermented by the "Shiga," "Kruse," or "New Haven" cultures, but by all others in twenty-four hours.

EXPERIMENTS WITH PEPTON NaCl WATER.

Composed of pepton, one per cent; NaCl, 0.5 per cent and distilled water, plus one per cent litmus solution. Sugars, one per cent.

In maltose "Kruse" gave acid in twenty-eight days; "Y" in twenty-one days; "Ferra," it is to be noted, gave acid in two days; the "Baltimore," "Flexner," and "Wollstein" cultures in one day usually. "Strong" showed no change after thirty-five days.

In saccharose "Y" gave acid in twenty-five days; "Diamond" and "Ferra" did not give acid. All the other organisms, with the exception of "Shiga," "Kruse," and "New Haven," which did not ferment saccharose, gave acid in from ten to fourteen days. "Seal Harbor" was not tested.

In dextrin the "Baltimore," "Gray," "Harris," and "Wollstein" cultures produced acid usually in twenty-four hours. "Ferra" in this test fermented dextrin in four to five days.

Dextrose and mannit were as usual. With the exception of the "Shiga," "Kruse," and "New Haven" cultures, which did not ferment mannit, acid was produced in all tests in twenty-four hours.

EXPERIMENTS WITH PEPTON ASPARAGIN WATER.

Composed of pepton, one per cent; asparagin, 0.5 per cent; and distilled water; one per cent of litmus solution, and sugars, one per cent. The medium is purple.

Here again in maltose we find "Kruse" giving rise to acid, commencing in nine days and increasing up to twenty-three days. "Y" commenced

to produce acid in eleven days; "Diamond" in nineteen days; "Ferra" in nineteen days. "Strong" did not ferment. The "Baltimore," "Philippine," and "Wollstein" cultures fermented the maltose usually in twenty-four hours.

In saccharose only "Ferra," twenty-three days, "Strong," nineteen days, and the "Baltimore," "Philippine," and "Wollstein" cultures fermented, these latter commencing on the sixth day and increasing up to the nineteenth day.

In dextrin, as usual, only "Baltimore," "Gray," "Harris," and "Wollstein" gave acid, one to three days.

Mannit and dextrose same as usual.

EXPERIMENTS WITH PEPTON UREA MEDIA.

Composed of pepton one per cent; urea, 0.5 per cent; distilled water, one per cent of litmus solution, and sugars one per cent. The medium is blue.

"Kruse" fermented maltose in nineteen to thirty-five days; "Y" and "Diamond" in twenty-three days; "Ferra" in twenty-seven days. "Strong" did not give rise to acid in thirty-five days. "Baltimore," "Harris," and "Wollstein" one day usually; "Gray" in five days.

The "Kruse" type did not ferment saccharose. "Y" gave some slight acid in thirty-five days and "Ferra" in twenty-seven days, "Strong" in fifteen days, and the "Baltimore-Harris" type in nine to eleven days.

Dextrin was fermented only by "Baltimore," "Harris," and "Wollstein" usually in one day; "Gray" in four days.

Mannit and dextrose as usual.

EXPERIMENTS WITH SUGAR-FREE BROTH.

Broth composed of Liebig's Extract, 5 grams; pepton, 10 grams; NaCl, 5 grams to 1,000 cubic centimeters distilled water. Subjected to fermentation with colon bacilli to free it from sugar. This was tinged with one per cent litmus solution. Sugars, one per cent.

In this medium fermentation was a little slower in dextrose and mannit than usual, full acid production requiring two days in dextrose, although change was always visible in twenty-four hours.

In the maltose "Kruse" showed acid production on the sixth day, increasing up to the fourteenth. "Y" did not ferment maltose in this experiment. "Diamond" commenced to produce acid on the sixth day, and "Ferra" on the twenty-first. "Strong" gave no acid. "Baltimore," "Harris," "Gray," and "Wollstein" showed acid reaction, one to two days.

In saccharose "Y" commenced to form acid on the twenty-ninth day and "Diamond" on the forty-first, "Ferra" on the thirteenth, "Strong" on the sixth, "Baltimore," "Harris," "Gray," and "Wollstein" on the sixth day.

In dextrin only the "Baltimore," "Harris," "Gray" and "Wollstein" cultures formed acid, as usual in twenty-four to forty-eight hours, with the exception of "Gray," which commenced on the third day and was complete on the eleventh.

EXPERIMENTS WITH NUTROSE MEDIA.

Nutrose, one per cent; normal sodium hydroxid solution, 0.4 per cent, and distilled water tinged with one per cent of the litmus solution. Sugar and mannit, one per cent. The hydroxid was added because the original solution was acid.

In these experiments with nutrose the full acid reaction is represented only by a change from purple to distinct red. A precipitate or complete coagulation of the medium did not occur in any test with dysentery bacilli, although the cultures were kept in the incubator for forty-five days.

All cultures fermented dextrose in this medium with marked acid production in twenty-four hours. No coagulum at any time. The same was true of the organisms fermenting mannit.

In maltose "Kruse" gave acid in ten days, increasing to fourteenth day. "Y" was slightly acid on twenty-eighth day; "Diamond" and "Ferra" on the twenty-fifth day. "Strong" did not ferment. "Baltimore," "Harris," "Gray," and "Wollstein" gave marked acid in twenty-four hours.

In saccharose "Ferra," fifteen days; "Strong," second day, and "Baltimore," "Harris," "Gray," and "Wollstein" commencing on third day were the only cultures to form acid.

In dextrin only "Baltimore," "Harris," "Gray," and "Wollstein" formed acid, showing faintly generally on second day, but "Gray" produced acid in this test even more slowly than usual.

EXPERIMENTS WITH LITMUS MILK.

As none of the cultures with which I was working was a lactose splitter, it occurred to me to use milk as a nutrient base for fermentation tests. The initial acid production due to the monosaccharid usually present was controlled by a series to which sugar had not been added. The milk was tinged with litmus, and sugars and mannit were added in one per cent proportion.

In the milk and dextrose "Kruse" produced distinct acid in forty-eight hours, and was coagulated in three days. "Y" coagulated in forty-eight hours, and also "Diamond" and "Ferra." "Strong" was fully coagulated in three days. "Baltimore" was coagulated on the fifth day and "Gray" on the sixth. These were the only cultures of the type tested.

In mannit, aside from the slight initial acidity, no change occurred in the "Kruse" culture until the eighth day, when a slight alkali production was manifest.

"Y," "Diamond," and "Ferra" coagulated the mannit milk inside of forty-eight hours. "Strong" turned it red by the third day, but the milk never coagulated. The "Baltimore" and "Gray" tubes were red on the second day, but not coagulated until the sixth day.

In maltose "Kruse" produced no change, becoming bluish on eighth day. "Y" and "Diamond" became alkaline on seventh day and distinctly blue on eighth, but changed again to red by last observation. "Diamond" eventually coagulated. "Strong" never changed from the initial acidity and did not become blue. "Ferra," "Baltimore," and "Gray" became markedly acid on third day, and brought about a coagulation about the seventh day.

In saccharose "Kruse" acted as in mannit and maltose; "Y" and "Diamond" as in maltose, but remained blue to the end of the observation. The "Ferra" tube was red on twelfth day, but coagulation did not take place even after two months. "Baltimore" became acid, but did not coagulate, and this was true of "Gray" and "Strong."

In dextrin "Kruse" acted as in mannit, maltose, and saccharose; in other words, as in ordinary litmus milk. "Y" and "Diamond" were blue again by eighth day and remained so throughout. "Ferra" became markedly acid, but did not coagulate; "Baltimore" and "Gray" became very acid, "Gray" finally coagulating on tenth day. "Strong" became distinctly blue by tenth day.

B. typhosus tested in the various media produced acid in dextrose, coagulating by twelfth day, and in mannit coagulating by twenty days. Acid formation took place in maltose and dextrin, but no coagulation occurred. The saccharose after initial reddening became purple again by sixth day and steadily more alkaline to end of observation.

In ordinary litmus milk my tests have all shown that after the initial acidity the organisms of the "Y" and "Baltimore-Philippine" types return usually by six or eight days to the original color, as do also the Shiga-Kruse cultures, but the "Y" and "Baltimore" types produce more alkali, approaching in this character more closely to the typhoid cultures, which, however, are more active alkaline producers than any dysentery organisms and eventually produce a distinctly different blueing of the cultures.

EXPERIMENTS IN NON-ALBUMINOUS SYNTHETIC MEDIA.

The medium employed was a modification of the Urschinsky-Fränkel medium.

Dipotassium phosphate	2 grams.
Magnesium sulphate	0.4 "
Sodium chlorid	5 "
Asparagin	4 "
Ammonium lactate	5 c.c.
Aqua. dest.	1,000 c.c.

The medium had an acid reaction to litmus (fifteen cubic centimeters of the five per cent solution) and six cubic centimeters $N/1$ NaOH were added, the color then being a purplish blue. The medium was clear. Sugars and mannit, one per cent.

Distinct acid was produced in all the dextrose cultures in from one to three days, and the same was true in mannit, with the exception, of course, of the Shiga-Kruse type cultures.

In maltose the "Y," "Diamond," and "Ferra" cultures gave rise to no acid, nor did the Shiga-Kruse cultures, even after thirty days at 37° C. "Strong" not only did not ferment the maltose with acid production, but the medium became distinctly bluer, and growth at the top of the medium, where oxygen was present, was abundant. Acid was rapidly (one to three days) produced in the "Baltimore," "Harris," "Gray," and "Wollstein" cultures.

In saccharose practically no change occurred in any of the cultures, with the exception of "Strong," which eventually produced distinct acid in thirty-one days.

In dextrin "Baltimore," "Gray," "Harris," and "Wollstein" gave rise to acid (one to three days). The other cultures, both of the Shiga-Kruse and Hiss-Russell type, eventually became distinctly bluer or alkaline.

Typhoid bacilli refused to grow in any of the media, the tubes remaining perfectly clear and unchanged.

With the exception of the culture in dextrose where acid was produced in quantity, the Shiga-Kruse cultures showed a distinct tendency to grow abundantly in the upper portion of the medium where free oxygen was present. The medium was favorable to the growth of all types of the dysentery bacilli. This, so far as my observation goes, would serve to differentiate them from the typhoid bacilli, which apparently do not multiply in this medium. The medium seems to be one to recommend for the study of this group.

ANAEROBIC FERMENTATION TESTS.

Properly speaking, the following tests are not anaerobic in the beginning, but serve the purpose of our experiment since the oxygen in the unfilled part of the tubes is exhausted by the initial growth of the organism.

Tubes containing serum water and the sugars, maltose, saccharose, and dextrin, were inoculated with the cultures to be tested, and then effectually sealed with sealing wax. Full series were not tested, only the three types of organisms represented by "Kruse," "Y," and the "Baltimore" cultures. "Ferra" was tested also because it approaches the "Y" culture most closely in the fermentations, yet does in some tests affect dextrin. The results are of interest, and "Ferra" is thrown distinctly in these experiments into the "Y" class.

In maltose all the cultures produce acid and eventually coagulate the medium, acid reaction being fairly early shown and coagulation taking

place in "Kruse" in twenty-one days, in "Y" and "Ferra" in twenty-two days, and in "Baltimore" in two days.

"Kruse" does not change the saccharose; "Y" and "Ferra" coagulate it only after thirty days. "Baltimore" shows a slight immediate change, but coagulates only after about thirty days.

In dextrin "Kruse" and "Y," and "Ferra" show no acid production. "Y" and "Ferra" after many days reduce the litmus, decolorizing the medium. "Baltimore," on the other hand, coagulates the medium in two days.

SUMMARY OF RESULTS OF FERMENTATION TESTS.—Our results may be summarized as follows: The organisms studied are divided fundamentally into two distinct groups by differences in their fermentation powers. The members of one of these groups do not, so far as can be determined, ferment mannit with acid production. The members of the other group produce acid from mannit. The first group, as is now well known, is composed of organisms corresponding to those described by Kruse as the cause of epidemic dysentery. Those studied by us are the "Kruse," "Shiga," and "New Haven" cultures, and the culture "Flexner" (from Manila?)*. The second group is represented by the cultures "Y" (Hiss and Russell), "Seal Harbor" (Park and Dunham), "Diamond" (Hiss), "Ferra" (Hiss), "Strong," Flexner's cultures "Harris" and "Gray" from Manila, "Baltimore" (Duval), and Dr. Wollstein's culture "Wollstein."

This second group, so sharply divided from the first by apparently a fundamental physiological difference, is itself, however, composed of organisms which are again separated into groups by differences which we have reason to believe are not, so far as fermentation goes, fundamental, but are, nevertheless, clearly marked.

The first of these groups in logical sequence is that represented by the Hiss and Russell organism "Y" as the type. This organism differs from the Kruse type by fermenting mannit. It has the ability, as we have seen, also to ferment

* Not mentioned in tests for fear of confusion, as the "Harris" and "Gray" cultures are generally referred to as Flexner organisms.

maltose and even saccharose. These fermentations, when they do occur, are, however, greatly delayed. To this class belong the cultures "Diamond," "Ferra," and "Seal Harbor."

The second group is represented by the culture "Strong." This organism ferments mannit rapidly, maltose very slowly, and saccharose readily.

The third group is represented by Flexner's Manila cultures "Gray" and "Harris," Duval's "Baltimore" culture, and "Wollstein." These organisms ferment mannit and also maltose, saccharose, and dextrin. These fermentations, with the exception of saccharose, are usually rapid, being distinctly manifest in twenty-four to forty-eight hours.

If we base a classification on characters determined say in a three to five day test, we may arrange our groups as follows:

"Shiga "	}	Ferment dextrose. Group I.
"Kruise" (type)		
"New Haven "		
"Y" (Hiss and Russell) (type)	}	Ferment dextrose and mannit. Group II.
"Seal Harbor "		
"Diamond "		
"Ferra "		
"Strong" (type) ferments dextrose, mannit, saccharose.		Group III.
"Harris" (type)	}	Ferment dextrose, mannit, maltose, saccharose, dextrin. Group IV.
"Gray "		
"Baltimore "		
"Wollstein "		
"B. typhosus "	{	Ferments dextrose, mannit, maltose, and dextrin. Typhoid Group.

These further studies have, therefore, practically substantiated the original classification made by me of these organisms in January, 1903, and have shown that the classification also holds for an increased number of cultures. The culture "Strong," which I thought at that time might possibly correspond to the type "Y," has, as we have seen, been shown to be of a different type. This could only be made out by tests extending over some days.

We have found, however, by our further studies that the Kruise type will after many days ferment maltose, and that

the "Y" type will eventually form acid from maltose and saccharose, and in one instance we find "Ferra" after many days fermenting dextrin. Evidences of these fermentations, however, appear very late, whereas in the case of the Baltimore-Flexner group the fermentation of maltose and dextrin is rapid, and that of saccharose takes place with regularity. The absence of signs of acid fermentation in maltose after one to five days is fair evidence of the organisms belonging to the "Y" or "Strong" type, but the absence or presence of fermentation in a pure dextrin is much more reliable, and is at present the surest fermentation test we have to identify the type of organism in the mannit-fermenting groups. The occurrence of such organisms as "Strong," however, makes a study in mannit, maltose, saccharose, and dextrin necessary.

ARTIFICIAL MODIFICATION OF FERMENTATIVE FUNCTIONS.

—Whenever fermentation unexpected from former tests occurred, as the delayed maltose and saccharose fermentations by members of the "Y" group, platings were made and the descendants of colonies tested for identity with the original organism and as to changes in rapidity of fermentation. The changes in fermentative power were frequently found to be marked, and were further increased by repeated transfers in the given maltose or saccharose medium. In the case of "Y" and "Diamond" and "Ferra," sub-cultures have been obtained which will produce marked acid from maltose and saccharose in twenty-four hours. Increase in the maltose-fermenting function carries no other fermentative change with it, but increase in the saccharose-fermenting power always carries increase in ability to split maltose, but not dextrin.

"Ferra," as we have seen, is erratic, but nevertheless belongs apparently with organisms of the "Y" group, although it is the only one so far known to ferment dextrin, no matter how prolonged the test. In the original culture the dextrin fermentation, if it occurs at all, is greatly delayed, while sub-cultures from such fermented dextrin tubes will, after a few generations in dextrin, ferment it readily inside of twenty-four

hours. The dextrin fermentation carries with it an increase in maltose-splitting power but not of saccharose, so that these artificial modifications never really bring the organism into conformity with the other group. We shall see, however, that these modifications of fermentative functions have some influence upon the agglutination reactions of the organisms. This will be reverted to in a subsequent paper.

STUDIES ON AGGLUTINATION. — As soon as I had determined by fermentation tests the apparent non-identity of the Flexner Manila and "Baltimore" cultures with the organisms of Shiga and Kruse and my culture "Y," I took up the careful comparative study of the agglutination reactions of all of these cultures in the presence of the serum of highly immunized animals and also in the serum of normal animals.

It is not my intention, however, to go into this subject in detail here, but simply to outline briefly the results of some of my earliest tests made during the first months of 1903.* The subject is treated fully in a paper by the writer and Dr. Charles Norris, which will shortly appear.

In most instances, although tests have been made with other cultures, types of organisms or groups will alone be referred to in the tables and text, Kruse's or Shiga's representing what we may refer to as Group I. ("Kruse," "Shiga," "New Haven,"), our organism "Y" representing Group II. ("Y," "Ferra," "Seal Harbor," "Diamond"),† and "Baltimore" representing Group IV. (the Flexner group — "Baltimore," "Harris," "Gray," "Wollstein"). "Strong" represents Group III., but was not fully tested for its serum reactions at this time.

Rabbits were used as the most convenient laboratory animals for immunization, and with few exceptions were successfully immunized. Organisms fermenting mannit being in the majority of instances not very pathogenic for these animals, their immunization is a simple matter. The true Shiga

* For some of the agglutination tests here given I am indebted to Dr. John C. Torrey, whose assistance I gratefully acknowledge.

† "Diamond" belongs to this group in fermentation reactions only; its agglutinations are different. See later paper by Hiss and Norris.

or Kruse organisms, on the other hand, are very pathogenic * for animals, and unless great care be taken they die from the introduction of either dead or living cultures. The most satisfactory method has been found to be the administration of very small primary subcutaneous doses of living cultures, allowing full recovery to take place before the second and increased dose is given. If this be done comparatively few animals are lost. In the immunization of all animals used in these tests only living agar cultures were administered. These were twenty-four hours old and emulsified in salt solution. In this way any objections and by-reactions that have been suggested as likely to be given by the use of bouillon have been avoided and eliminated.

The agglutination results were obtained by macroscopic tests in small test-tubes. Emulsions of agar cultures, twenty-four hours old, in 0.85 per cent of NaCl solution, ten cubic centimeters to a culture, were always used. In the tubes the quantity of culture and serum was always made up to two cubic centimeters — one cubic centimeter of the serum dilution and one cubic centimeter of the salt emulsion. Twenty-four hours was the limit of the test. The results, determined by the use of a hand lens, were as follows:

Rabbit immunized against Group I. (Shiga's culture).

Bacilli of Group I.:

"Shiga" (homologous)	20,000
"Kruse"	20,000
"New Haven"	20,000

Bacilli of Group II.:

"Y"	200
"Ferra"	200
"Seal Harbor"	200

Bacilli of Group IV.:

"Baltimore"	800
"Harris"	800
"Gray"	800
"Wollstein"	800

Typhoid bacilli	20
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* The difference in pathogenicity, as evidenced by the symptoms of paralysis or weakness apparent in the posterior extremities of rabbits, is really sufficient to separate these organisms from the mannit-fermenting variety.

Rabbit immunized against Group II. ("Y" culture, Hiss and Russell).

Bacilli of Group I.:

"Shiga"	less than	100
"Kruise"	" "	100
"New Haven"	" "	100

Bacilli of Group II.:

"Y" (homologous)	6,400
"Ferra"	6,400
"Seal Harbor"	6,400

Bacilli of Group IV.:

"Baltimore"	1,600
"Gray"	1,600
"Harris"	1,600
"Wollstein"	1,600
Typhoid bacilli	100

Rabbit immunized against Group IV. ("Baltimore" culture).

Bacilli of Group I.:

"Shiga"	less than	100
"Kruise"	" "	100
"New Haven"	" "	100

Bacilli of Group II.:

"Y"	400
"Ferra"	400
"Seal Harbor"	400

Bacilli of Group IV.:

"Baltimore" (homologous)	3,200
"Harris"	3,200
"Gray"	3,200
"Wollstein"	3,200

It is thus readily seen that the organisms belonging to the groups differentiated by fermentation tests are also distinctly separated by marked differences in their agglutination limits, and that careful agglutination tests in the serum of immunized animals shown not to have high normal agglutinating power for any members of the group would not have failed to call attention to the differences in these organisms, and should have prevented a recurrence of such confusion in the identification of these forms as has often been the case between the true Shiga organism and the mannit fermenters in general.

That the organisms forming the groups or types of mannit fermenters have some agglutinable substances in common or which fix the same agglutinins and give rise to similar or the same agglutinins in inoculated animals is true, but the marked differences in the limits of the agglutination shown by all organisms that differ in their fermentation reactions is very significant.

If the normal serum of such an animal as the beef or goat be examined, it is often found to contain agglutinin for these mannit-fermenting organisms. But here again if the limits of agglutination are determined, a marked difference is generally found in the reaction of the different groups.

Thus, a test of the agglutination of members of the various groups in normal beef serum gives the following results:

Group I.	20 or less
Group II.	100
Group IV.	200
Group typhoid	100

These results in beef serum are confirmed by many tests, some of which were mentioned in our previous article.

In the case of goat's serum we find the following:

Group I.	(No test)
Group II.	200
Group IV.	400
Group typhoid	40

The proportional difference between Groups II. and IV. is the same as in beef serum; typhoid agglutinins are, however, in different proportion.

ABSORPTION TESTS.—To test the specificity of these reactions in immunized sera and normal sera, and to be sure that they were not simply due to differences in the number of receptors or absorbing power of the different cultures instead of being actually different, absorption tests were made. These were carried on carefully, and as complete an absorption obtained as possible. Usually, one cubic centimeter of normal or one-fifth cubic centimeter of the immune

serum to be exhausted of a given agglutinin was placed in a graduated centrifugalizing tube, and agar cultures of the bacillus, agglutinins for which were to be exhausted, emulsified each in one cubic centimeter of NaCl solution, were added until the serum fluid remained permanently cloudy. Often eight or nine cultures were required for complete exhaustion, and the resulting fluid representing a 1 to 10 or 1 to 50 dilution of the serum was then carefully centrifuged and the clear fluid used in the tests. Usually in the serum of immunized animals agglutinations below 1:50 or 1:100 were disregarded, and such reactions are generally represented as 0. The results of these tests may be briefly given as follows:

The Shiga serum given above may be taken as an example.

Group I., immune serum (Shiga's cultures).

(a.) Agglutinins removed by saturation with a Group I. culture (Shiga culture):

	After saturation.	Before saturation.
Agglutination of Group I.	0	20,000
" " " II.	0	200
" " " IV.	0	800

(b.) Saturation with a Group II. culture (Bacillus "Y"):

	After saturation.	Before saturation.
Agglutination of Group I.	20,000	20,000
" " " II.	0	200
" " " IV.	400	800

(c.) Saturation with Group IV. cultures ("Baltimore"):

	After saturation.	Before saturation.
Agglutination of Group I.	20,000	20,000
" " " II.	200	200
" " " IV.	0	800

Here we find that bacilli of Group I. absorb all the agglutinins from Group I. serum which have been developed by the immunization. Below 1:100 some of the normal agglutinins may come into play, hence such agglutinations are disregarded. The reason for this will be apparent later from the experiments with normal sera.

In the case of absorption by Group II. we find, however, none of the agglutinins for Group I., which affect the limits

of Group I. agglutination removed. All of those for Group II. have been absorbed, and some of the agglutinins affecting Group IV. have also been removed. Hence, two agglutinins appear to play a part in determining the agglutination limit of Group IV. in Group I. serum.

Again, when the agglutinins for Group IV. have been removed we find the amount of Group I.'s agglutinins unaffected. Group II.'s agglutinins have also apparently not been reduced, showing thus no agglutinin in common with Group IV. which affects Group II.'s limit of agglutination.

These researches are instructive, but have only a passing interest, as *B. dysenteriae* and mannit fermenters are already fully recognized as distinctly different in their agglutination reactions. The differences between Groups II. and IV. are, however, of much importance, as these organisms are usually looked upon as one and the same, and practically little heed seems to have been given to the differences in agglutination shown by Kruse and to the cultural differences already pointed out by us.

Group II., immune serum ("Y" culture).

(a.) Group I. agglutinins removed by saturation with Shiga's culture:

	After saturation.	Before saturation.
Agglutination of Group I.	0	100 (less)
" " " II.	6,400	6,400
" " " IV.	1,600	1,600

(b.) Group II. agglutinins removed by saturation with "Y" culture (also "Ferra"):

	After saturation.	Before saturation.
Agglutination of Group I.	0	100
" " " II.	0	6,400
" " " IV.	0	1,600

(c.) Group IV. agglutinins removed by saturation with "Baltimore" culture:

	After saturation.	Before saturation.
Agglutination of Group I.	0	—100
" " " II.	6,400	6,400
" " " IV.	0	1,600

We find, as was to be expected, as a result of the Group II. saturation all the agglutinins absorbed not only for Group

II. but for Groups I. and IV., as these agglutinins were called forth by inoculations with Group II. bacilli. When we exhaust by absorption, however, with Groups I. and IV. bacilli the results are different.

The agglutinins determining the maximum limit of Group II.'s agglutination are "specific," and not fixed and removed by Groups I. and IV. organisms. When we perform the same experiment with Group IV. serum, similar results are obtained.

Group IV., immune serum ("Baltimore" culture).

(a.) Group I. agglutinins removed by saturation with Shiga's culture:

	After saturation.	Before saturation.
Agglutination of Group I.	0	—100
" " " II.	400	400
" " " IV.	3,200	3,200

(b.) Group II. agglutinins removed by saturation with culture "Y":

	After saturation.	Before saturation.
Agglutination of Group I.	—100	—100
" " " II.	0	400
" " " IV.	3,200	3,200

(c.) Group IV. agglutinins removed by saturation with culture "Baltimore":

	After saturation.	Before saturation.
Agglutination of Group I.	0	—100
" " " II.	0	400
" " " IV.	0	3,200

Saturation with Group IV. bacilli removes all agglutinins. After saturation with Group II. bacilli the major agglutinins of Group IV. remain intact, while the minor agglutinins caused by the Group IV. inoculations and which affect Group II. are removed. In the case of Group I. absorption the picture is similar, the limits of Groups II. and IV. remaining unchanged.

It is plain, then, from these experiments that the major or chief agglutinins developed in each case in the homologous serum are specific, and that the increase in agglutinating value of the serum of an immunized animal for other organisms is due to the minor ("neben" or "partial") agglutinins arising from inoculation with any of these organisms and

which are fixed in common by them, and may perhaps be looked upon as indicating a more or less remote phylogenetic relationship of such organisms. Possibly they are to some extent agglutinins which would even be developed by inoculations with many organisms of very distant relationships and even of different classes.

In this connection experiments carried on with typhoid serum may be instructive. The serum of a rabbit highly immunized to typhoid bacilli showed the following agglutination:

Group II. (agglutination)	600
Group IV. "	1,500
Typhoid (homologous agglutination).	50,000

Typhoid serum. Absorption of Group II. agglutinins:

		Normal.
Group II. (agglutination)	0	600
Group IV. "	400	1,500
Typhoid "	50,000	50,000

Thus we find the typhoid limit unaffected, but that some (two-thirds) of the agglutinins for Group IV. have been removed as well as all the Group II. agglutinins. This shows that Groups II. and IV. have common receptors for some of the minor agglutinins developed under the influence of the typhoid bacillus.

Typhoid serum. Absorption of Group IV. agglutinins:

		Normal.
Group II. (agglutination)	200	600
Group IV. "	0	1,500
Typhoid "	50,000	50,000

The typhoid limit or, in other words, the major agglutinins are unaffected, while some of the minor agglutinins affecting Group II. are removed. In this case two-thirds of Group II. agglutinins are absorbed by Group IV.

This study of the typhoid serum shows that the minor agglutinins developed in an animal by such an organism as the typhoid bacillus, and which are able to agglutinate members of Groups II. and IV. of the dysentery organisms, are

not necessarily specific for either of these groups, but are capable of uniting with receptors of bacilli of both, and that the saturation with bacilli of one group will affect the limit of agglutination of the members of the other group; hence these agglutinins, in part at least and probably in entirety since the typhoid bacillus removes them, differ from the major or "specific" agglutinins called out by an inoculation or infection with either one of these bacilli. This further demonstrates plainly what is already known—that inoculation with a given species of bacteria calls forth more than one kind of agglutinin, and that these are, it seems, of a more generalized type than the major or chief agglutinins of each species which seem to be specific for the special species used; for these minor agglutinins seem to have the power of uniting with the more primitive or racial receptors of the bacteria brought into their presence, while the major agglutinins are more truly specific for the homologous organisms.

The careful study of the action of agglutinins of normal sera, such as those of the beef and goat, is also instructive, and really serves to differentiate the various members of different groups of organisms from each other quite as well as the study of the agglutinins of specific sera. Our attention has especially centered on beef serum, and interesting results have been obtained. Thus, as cited before:

Agglutination tests in normal beef serum:

Group I. (agglutination)	20
Group II. "	100
Group IV. "	200
Typhoid "	100

Normal beef serum. Absorption of Group I. agglutinins:

		Normal.
Group I. (agglutination)	0	20
Group II. "	100	100
Group IV. "	200	200
Group Typhoid "	100 (slight)	100

Normal beef serum. Absorption of Group II. agglutinins:

		Normal.
Group I. (agglutination)	20	20
Group II. "	0	100
Group IV. "	100	200
Group Typhoid "	50	100

In this case the removal of the normal agglutinins affecting Group II. ("Y") also reduces the limit of Group IV. one-half and of typhoid one-half. When, however, we try

Normal beef serum. Absorption of Group IV. agglutinins:

		Normal.
Group I. (agglutination)	20	20
Group II. "	100	100
Group IV. "	0	200
Group Typhoid "	50	100

we find the limit of agglutination of Group II. undisturbed, showing that the agglutinins affecting Group IV., although in part absorbed as we have just seen by Group II., are not those that necessarily determine the limit of agglutination of the Group II. organisms. The typhoid agglutinins have again been reduced one-half.

Normal beef serum. Absorption of typhoid agglutinins:

		Normal.
Group I. (agglutination)	20	20
Group II. "	100	100
Group IV. "	200	200
Group Typhoid "	0	100

Only the typhoid agglutination is affected by the absorption of the normal agglutinin having an affinity for the typhoid bacillus. This shows that Groups II. and IV. have receptors for agglutinins of normal sera which affect the typhoid bacilli, but that agglutinins determining the limits of agglutination of these groups themselves are not fixed by the typhoid bacillus. These results further indicate that the agglutinins absorbed by Groups II. and IV. from normal beef serum are not the same as those minor agglutinins that would be developed in the serum of an animal immunized against typhoid bacilli, since the typhoid bacillus cannot remove them. The agglutinin or agglutinins in normal serum which affect the typhoid bacillus itself, and also the Groups II. and IV., may very probably represent the minor agglutinins, the production of which is stimulated during a typhoid immunization. That they are not the same as the major typhoid

agglutinins is shown by their being absorbed by Groups II. and IV., which, as we have seen, is not the case with the major agglutinin for the typhoid bacillus. The same is true of some at least of the normal agglutinins which affect Group IV., since one-half of them are removed by absorption with bacilli of Group II. Whether the other half is the specific agglutinin it is at present impossible to say. It is interesting, however, to note in this connection that in the rabbit serum immunized to typhoid, Group II. bacilli absorbed two-thirds of the agglutinins affecting Group IV., and that Group IV. absorbed about two-thirds of those determining the limits of Group II.'s agglutination; both of these being, of course, minor agglutinins of typhoid, it is not unlikely that they correspond to some of the minor agglutinins affecting the typhoid bacillus in beef serum.

GENERAL CONCLUSIONS.

The results of our study indicate that the bacilli isolated from cases of dysenteric and diarrheic diseases, so far as the organisms that have come into our possession are concerned, fall into four major groups which have fermentative and agglutinative characters distinguishing them from one another and from the bacillus of typhoid fever. Thus, they may be differentiated by their growth in sugar media and by marked differences in their agglutinative characters in various sera.

The grouping of the different organisms according to differences in fermentative characters corresponds to that resulting from determinable differences in agglutinative action in specific sera. Sub-groups, however, due to differences in agglutinative characters alone may occur among cultures having, so far as determined, the same fermentative functions.

The groups as determined by fermentation tests are as follows: The first, represented by *B. dysenteriae* of Shiga and Kruse, ferments monosaccharids readily, and at times, after many days, maltose. This group is distinguished from all of the following groups by not fermenting mannit. The second group, represented by our bacillus "Y," ferments

monosaccharids and alcohol mannit generally within twenty-four hours. Maltose (and at times, under special conditions, saccharose also) may be fermented, but not with ease. The third group, represented by Strong's Philippine culture, ferments monosaccharids and mannit with ease; saccharose is fermented comparatively readily, and at times maltose, but slowly. The organisms composing the fourth group, represented by Flexner's Manila cultures and Duval's "Baltimore" culture, ferment monosaccharids, and mannit, maltose, saccharose, and dextrin with ease, although the saccharose fermentation is usually less complete and slower than the others. Typhoid bacilli ferment monosaccharids, mannit, maltose, and dextrin, and are distinguished from the last group by not fermenting saccharose and also by their motility.

Agglutination tests show a wide difference in the agglutinative characters of these different groups; this is apparent from their reaction in specific as well as in normal sera.

Absorption tests demonstrate that the major ("chief," "haupt") agglutinins developed in the serum of animals inoculated with representatives of these groups are apparently distinct and specific, and are only removed by saturation of the serum with the homologous organism or one of its groups or species. By such a saturation the minor ("neben" or "partial") agglutinins are also removed.

The organisms belonging to the heterologous groups simply absorb the minor agglutinins, but do not diminish the major agglutinins which determine the limit of agglutination of the homologous organism or those grouped with it by fermentation tests.

The agglutination tests are thus found to support and possibly to extend the classification indicated by differences in fermentative activities, and not only are the Shiga-Kruse bacilli, as is already fully recognized, differentiated from the mannit-fermenting organisms, but these latter are shown to be composed of several well marked and easily distinguishable varieties, if not indeed distinct species.

What the etiological significance of these various organisms is has not as yet been satisfactorily determined. So

far as their occurrence in abundance in the digestive tract, coincident with the development of certain inflammatory conditions and the increase of apparently specific agglutinins in the blood of the patient, goes, they all at present, it seems, have an equal claim to be looked upon as possible inciters of dysenteric and diarrheic diseases. Whether one or all, or indeed any of them, will continue to hold this claim only further observation and experimentation can determine.

So much attention is now being paid to intestinal bacteria in disease that many organisms hitherto undescribed or imperfectly known are being recognized, and there is a tendency hastily to associate them etiologically with the pathological condition of the intestinal tract. But so many factors may play a part in disturbances of the digestive functions and inflammations of the digestive tract that definite conclusions in regard to etiological significance are not entirely warranted when based upon the mere presence of hitherto undescribed or little known organisms in large numbers, even when these are associated with inflammatory conditions and a rise of agglutinating power in the blood of the patient. For it must still be kept in mind that the organism may be present on account of the condition, rather than as an original inciting factor.

It seems, therefore, that future endeavor should be directed to the careful investigation, morphological, bacteriological, and clinical, of extended series of cases to determine, if possible, whether the pathological picture or the clinical type of the disease varies definitely with the type of supposed infecting organism.

The marked pathogenicity of the Shiga-Kruse organisms as compared with that of the mannit-fermenting organisms, and the difference in the specific reactions called forth in the animal body by these various organisms, make it not improbable that if they are true etiological factors, detectable differences may exist in the clinical or pathological picture induced by them, and such differences thus aid in the fuller recognition and establishment of the etiological significance of the organisms concerned.

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THE DIFFERENTIATION OF BACILLUS DIPHTHERIÆ, BACILLUS XEROSIS, AND BACILLUS PSEUDO-DIPHTHERIÆ, BY FERMENTATION TESTS IN THE SERUM-WATER MEDIA OF HISS.*

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The differentiation of the true diphtheria bacillus from bacilli which closely resemble it except in virulence has been the subject of considerable study in recent years on account of its importance from a clinical and biological standpoint. These similar bacilli are:

I. The xerosis bacillus, which was first described by Kutschbert and Neisser¹ in 1883. It has since become recognized as an almost constant inhabitant of the conjunctiva in health and disease. Schanz,² Pes,³ Peters,⁴ and others regard the Bacillus xerosis and Bacillus diphtheriæ as identical, the xerosis bacillus being a non-virulent variety of Bacillus diphtheriæ. Axenfeld⁵ and Franke⁶ are of an opposite opinion.

II. The pseudo-diphtheria bacillus, which was first described by Hoffmann-Wellenhof⁷ and by Lœffler⁸ in 1887. It was found in the throats of non-diphtheritic subjects, and resembled the diphtheria bacillus, but was non-virulent. Roux and Yersin⁹ regarded it as a non-virulent diphtheria bacillus.

Many laborious investigations have shown some points of dissimilarity in certain cultures of these organisms, in the morphology, the reaction to special stains, acid production, and growth on various media. These differences are, however, not constant, so that at present it is generally accepted that these bacteria cannot, with certainty, be distinguished morphologically or culturally from one another.

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In 1897 M. Neisser¹⁰ described a method of staining by which, under special conditions, *Bacillus diphtheriæ* showed typical granules, which were said to be absent in *Bacillus xerosis*. It now seems that this is not the case, the granules being demonstrable in the xerosis bacilli also, but at a later period of their cultural development (Schanz¹¹). Heinersdorf,¹² however, never obtained a double stain characteristic of true diphtheria bacilli in sixty cultures of *Bacillus xerosis* of less than twelve hours' growth. In animal inoculations with cultures and diphtheria anti-toxin (Spronck,¹³ quoted from Denny¹⁴) the lesions regularly produced by the slightly virulent diphtheria bacilli were prevented, while in the case of inoculations of *Bacillus pseudo-diphtheriæ* and *Bacillus xerosis* the production of lesions was not prevented.

Denny studied the changes in the morphology of these three species during their growth on blood serum, and also the influence exerted on the morphology by variations of temperature, reaction of media, and symbiosis, and found that these species were always indistinguishable morphologically at certain stages and under certain conditions. He was, however, able to find distinguishing characteristics if all parallel developmental stages were observed.

Schwoner¹⁵ succeeded in differentiating the diphtheria bacillus and the pseudo-diphtheria bacillus by agglutination in specific sera.

The determination of the pathogenicity of these bacilli, and the toxicity of their culture fluids is, however, generally considered the final and only reliable means of differentiation.

PERSONAL EXPERIMENTS. — The large numbers of xerosis bacilli encountered in a study of conjunctival lesions which was being carried on by the writer led, at Dr. Hiss' suggestion, to an investigation of the ability of *Bacillus xerosis* to ferment sugars with acid production and to a comparison of its fermentative powers with those of the true bacillus diphtheriæ and *Bacillus pseudo-diphtheriæ*.

The method of study followed has been employed by Hiss

in differentiating the pneumococcus and streptococcus (Centralblatt f. Bakt., 1902), and more recently the organisms of dysentery and typhoid fever (Medical News, Feb. 14, 1903). This method consists in the use of serum-water media. The medium is composed of beef serum, 1 part, distilled water, 3 parts, and is practically sugar free. After heating to 100° C. for a short time to destroy the enzymes of the blood, one per cent additions of the following sugars are made: dextrose, mannite, maltose, lactose, saccharose, and dextrin. Finally litmus solution (Merck's pure litmus, five per cent solution in water) is added in the proportion of one per cent. The media are tubed and sterilized at 100° C. for ten minutes on three consecutive days.

The growth of organisms in these media may, according to their physiological peculiarities, be associated with the production of acid, a consequent reddening, and coagulation of the media containing certain of these sugars. Thus, if different sugars are affected, a means of differentiation is furnished.

By this method twenty-seven cultures of diphtheria bacilli, ten cultures of xerosis bacilli, and four cultures of pseudo-diphtheria bacilli were tested.*

The following constant results were obtained after twenty-four to forty-eight hours' growth at 37° C.

Pseudo-diphtheria bacillus: None of the sugars were fermented; the media remained blue.

Diphtheria bacillus: Dextrose, mannite, maltose, and dextrin were fermented with acid production, the medium becoming red and coagulating. Saccharose was not fermented.

Xerosis bacillus: Dextrose, mannite, maltose, and saccharose were fermented with acid production; the medium turned red and coagulated. Dextrin was not fermented.

These results do not change after many days at 37° C.

A peculiarity of the growth of the xerosis bacillus was the formation of a very thin scum or pellicle on the surface of the media. This was absent in the cultures of the two other species.

*For some of these cultures I am indebted to Dr. W. H. Park of the New York Department of Health, and to Dr. Mary Heffernan of the University of Chicago.

CONCLUSIONS. — We thus see from these experiments that the diphtheria culture and the cultures of *Bacillus xerosis* ferment dextrose, mannite, and maltose. Their behavior in the presence of these carbohydrates offers, therefore, no point of differentiation.

The results with saccharose are, however, different. The xerosis bacilli ferment saccharose, while the true diphtheria bacillus, so far as our experience goes, does not.

In the case of dextrin we also find a difference. Dextrin is fermented by the diphtheria bacilli, but not by the xerosis bacilli.

Pseudo-diphtheria bacilli do not ferment any of the sugars tested. Hence a study in saccharose and dextrin media will, it seems, serve to differentiate these three types or species of organisms with which we have experimented. If the organism does not ferment either of these sugars, it is the pseudo-diphtheria bacillus; if it ferments saccharose, it is the xerosis bacillus; if dextrin is fermented, it is the true diphtheria bacillus.

Although the series of experiments on which this differentiation is based is not extensive enough to be exhaustive, still the results have been so uniform that we feel justified in calling attention to them as indicating a method of differentiation, which, it is hoped, may prove as reliable as it is simple and rapid.

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A CASE OF PERNICIOUS ANAEMIA,
SHOWING A MEGALOBlastic CRISIS FOLLOWED BY
MARKED IMPROVEMENT.

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The following case of pernicious anaemia is one that for a time showed progressive changes for the worse, marked especially by increase in the number of megaloblasts. Under treatment of a few weeks there was a total disappearance of the nucleated cells and a remarkable change for the better in the condition of both the blood and the patient.

The patient was a German woman of fifty-two years of age, who came to this country when sixteen. She was married when twenty-four and had had four children and several miscarriages. Until within a year she had enjoyed good health, but had always worked hard and two years ago had nursed her husband through a long illness. Twelve months ago she began to feel run down and she said people told her that her blood was poor. She suffered from dyspnoea and constipation, and a month before coming to the Vanderbilt Clinic her feet began to swell and she became very nervous. She was living in a damp ground-floor flat, practically alone, doing all her own work up to the time of going to the hospital.

Physical Examination showed a large, rather well nourished woman with pale mucous membranes and a yellow tinge to the skin, but not the color that would

suggest an advanced pernicious anaemia. In fact the diagnosis was not made until the blood was examined. There was a loud systolic murmur, loudest over the pulmonic area, and marked oedema of the legs and ankles. The urine showed a trace of albumin and hyaline casts.

For a period of ten days she was able at intervals to come to the Clinic from Harlem, and she got some relief from the bandaging of her legs and feet, but was unable to take food or keep warm. The dyspnoea increased and she grew weaker, until finally on November 11, she collapsed and had to be helped across the street to the Hospital. She had then developed a bad bronchitis with considerable temperature.

For the opportunity to make the subsequent observations I am indebted to Dr. Walter B. James, to whose service she was admitted at the Roosevelt Hospital.

Blood Examinations:

The observations on the blood were made during a period of ten weeks. The morphology was studied from smears stained with Wright's chromatin stain. This stain is the most satisfactory for pernicious anaemia, as it brings out far better than others the difference between megaloblasts with polychromatophilia and large lymphocytes. With Jenner's stain it is often very hard to differentiate between the two. Wright's stain also gives a lighter, cleaner stain to the body of the red cell, which makes it preferable also for studying chlorosis and secondary anaemias.

The number of megaloblasts in the blood on different days was estimated in two ways.

1. They were counted during a differential count of the leucocytes and their number to the cubic millimeter estimated by subtracting from the leucocyte count,

DATE	HbGLOBIN.	RED CELLS.	WHITE CELLS.	POLYNUCLEAR NEUTROPHILS.	LARGE LYMPHOCYTES.	SMALL LYMPHOCYTES.	EOSINOPHILS.	MAST CELLS.	MEGALO-BLASTS.		NORMO-BLASTS.		ESTIMATED NUMBER OF MEGALOBLASTS TO C.M.M.	ESTIMATED NUMBER OF NORMOBLASTS TO C.M.M.
									PER CENT.	WHITE CELLS IN COUNT.	PER CENT.	WHITE CELLS IN COUNT.		
Oct. 30	25	1,152,000	11,000	59.	8.	30.8	2.2		3.8	21	2.4	12	342	216
Nov. 4	20	984,000	6,000 ?	50.8	10.6	36.4	2.2		5.2	27	1.6	9	312 ?	96 ?
Nov. 11	15	968,000		72.6	8.2	18.4	0.6	0.2	17.	92	2.6	16	986	150
Nov. 12			5,800	72.4	12.2	14.6	0.2	0.6	7.6	40	4.6	25	440	266
Nov. 16				77.	11.8	9.2	1.8	0.2	17.2	108	7.8	45	997	462
Nov. 27	30	1,100,000												
Nov. 28			7,800	88.6	7.	4.	0.4	0.	0.		0.			
Dec. 3	35	1,400,000	7,800											
Dec. 8			11,000	79.6	7.2	7.6	5.6		0.		0.6	3		
Dec. 19	47	3,240,000	8,000											
Jan. 7	55	3,000,000	6,500	74.6	10.	10.6	4.2	.6	0.		0.			

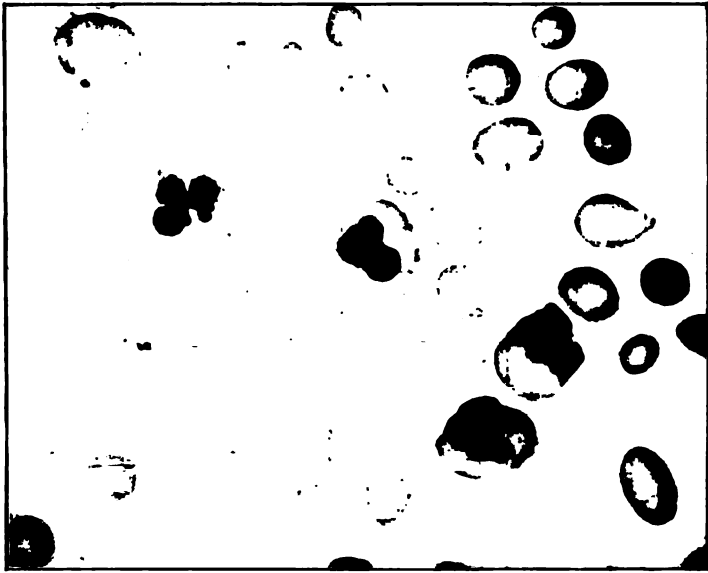


FIG. 1.—Smear taken on November 11, showing group of megaloblasts.

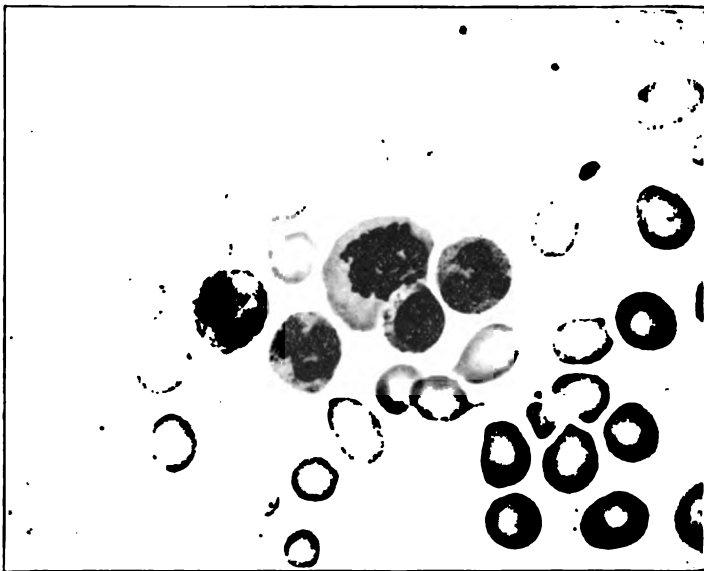


FIG. 2.—Smear also of November 11. Megaloblast showing mitosis in center of group of polynuclear neutrophils. A lymphocyte overlies the megaloblast.

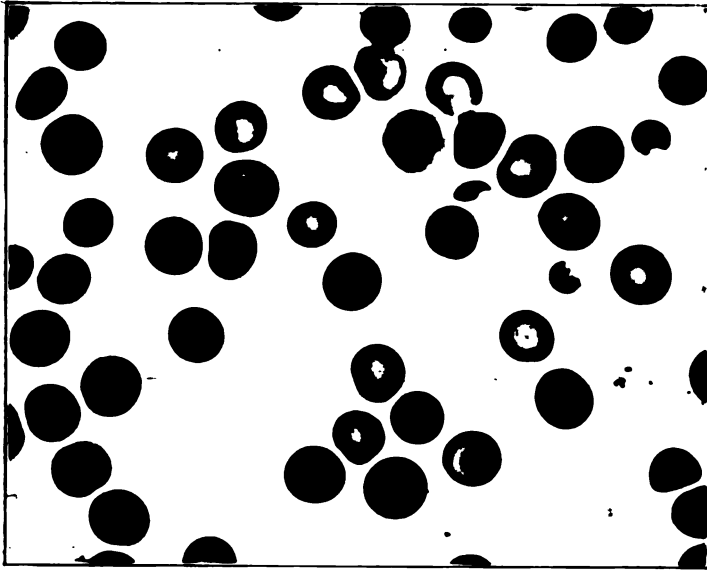


FIG. 8.—Smear taken January 9. Lymphocyte in upper part of field. Nearly all the red cells are normal in size and shape.

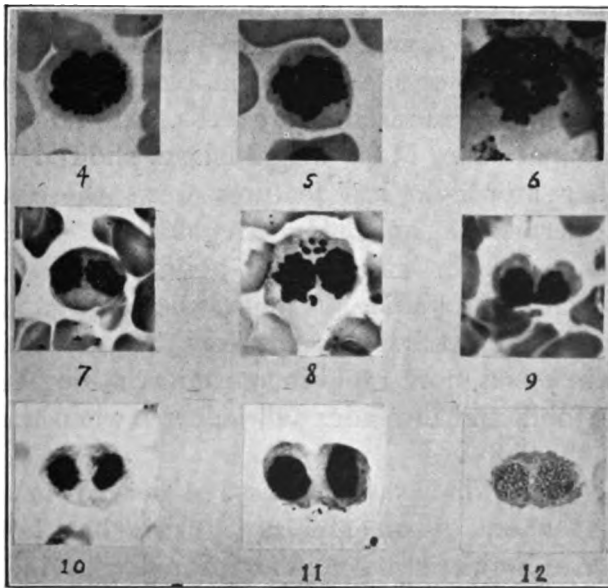


FIG. 4-12.—Megaloblasts showing various stages of mitosis.

The photographs were taken by DR. E. LKAMING, and with the exception of Fig. 12, the magnification is 1145 diameters.

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inasmuch as all nucleated cells are counted in the white cell count. On those days when a differential count was made but no leucocyte count, the number of leucocytes was assumed to be about the same as the count on a nearby date.

2. In making a differential count of five hundred white cells note was made of the number of megaloblasts seen.

The number of normoblasts was estimated in the same way. Microblasts were not seen at any time. The accompanying table gives the cell counts, hemoglobin, differential count, and the number of nucleated red cells. The megaloblastic crisis appears to have occurred between November 11 and 16, when the number per cubic millimeter ran up to nine hundred and ninety-seven. This is some three hundred more than Da Costa found in the highest count in his series of twenty-nine cases. Twelve days later careful search failed to show any nucleated cells. On December 8, three normoblasts were seen in counting the five hundred white cells, but none in the later examinations. Many megaloblasts showed mitosis. The other morphological features were those usually seen in pernicious anaemia. Megalocytes were very numerous and there was marked poikilocytosis. There was polychromatophilia of both nucleated and non-nucleated cells. At first the cells were rich in hemoglobin; later there was a more rapid increase in cell number than in hemoglobin and the index fell below 1. when the count reached 3,000,000.

The case follows the course observed in several instances where a megaloblastic crisis has been the precursor of an increased number of red cells indicating great activity of the bone marrow. The lymphocytosis is most marked before the crisis when the percentage of

A CASE OF PERNICIOUS ANAEMIA.

large and small reaches forty-seven. The eosinophiles and mast cells are within normal limits and no myelocytes were observed.

With the changes in the blood there was steady improvement in the condition of the patient. The severe attack of bronchitis developing when the blood was so impoverished (red cells below 1,000,000 and hemoglobin below 20%) had given a bad prognosis, but when this left her the dyspnoea decreased, the oedema of the legs went down and for many days before leaving the hospital she was able to walk about the ward. The treatment throughout was with Fowler's solution in increasing doses up to nine drops three times a day.

The last smears of the blood were made on January 9, the day before she left the hospital. The contrast between those and the earlier smears was very great. An occasional megalocyte or poikilocyte was the only thing to suggest that the blood was not normal.

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DRY IODINE CATGUT.¹

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ABOUT one year ago I presented to the Surgical Section of the New York Academy of Medicine a brief report¹ on Claudius's catgut, in which I pointed out its advantages over catgut prepared by other methods. This method of preparation was soon adopted by surgeons in various parts of the country, and it was not long before I received various complaints concerning its tensile strength; and it is but proper to confess that, though we continued to use it, we also found that this complaint was not entirely without foundation. Its other advantages were, however, so preponderating, that, though not satisfactory in all details, we were loath to abandon it entirely, and I therefore set myself the task to find, and if possible to eliminate, the causes of this loss of strength. I soon became convinced that this was due only to its long-continued immersion in the iodine solution. In other words, no change was necessary in the preparation of the catgut, but one was indicated in its subsequent preservation. According to Claudius's directions, the catgut was preserved in the original iodine solu-

¹ Read before the Surgical Section of the New York Academy of Medicine, May 5, 1905.

tion for an indefinite time. I have changed these directions in so far that I remove the catgut from the iodine solution at the end of eight days, and thereafter keep it dry and ready for use in a sterile vessel. This step, however, be it ever so simple, involved to my mind a material departure from Claudius's method, and it was necessary therefore to again subject the dry catgut to an investigation of the attributes required of good catgut; and it is the object of this paper to present the results of my research.

As I pointed out in my original article, the four cardinal attributes which must be present in an ideal catgut are, (1) It should be absolutely sterile; (2) in the course of preparation it should not lose any of its tensile strength; (3) it should be readily and simply prepared, and without any undue expense, and (4) it should be absorbed completely, but only after it has served the purposes for which it was intended.

I. STERILITY.—Dr. Arpad G. Gerster and myself have used in the First Surgical Division of Mount Sinai Hospital as a routine procedure catgut prepared by means of iodine solution for nearly three years; during the past nine months after the modified method; and in no case have we had the slightest occasion for regretting our confidence in it. What is of practical interest at the present time is our experience with the newer catgut, and I can only say, that during the time mentioned we have used it in every instance where the use of catgut was indicated, not only as ligature, but also as suture material, and at no time did we see any untoward effects from it.

As already stated, the method now in use is to my mind an important departure from the original method of Claudius. Sufficient evidence for the sterility of catgut prepared by the Claudius method was presented by Claudius,² ³ Martina,⁴ von Hippel,⁵ and Johnson;⁶ but it was necessary to examine the newer catgut also from a bacteriological stand-point. A few preliminary experiments were made for me by Dr. Alfred Cohn, of the house staff of Mount Sinai Hospital; the detailed experiments I was able to conduct at the Pathological Laboratory of the College of Physicians and Surgeons, Columbia Uni-

versity, through the courtesy of Dr. T. Mitchell Prudden; and I take great pleasure on this occasion to express my gratitude to Dr. Prudden, not only for the many courtesies extended, but also for his valuable advice and for the interest shown in my work.

At the outset, I would state that all of the experiments were conducted with No. 1 iodine catgut, and that all of the experiments were controlled with catgut of similar size, prepared after the von Bergmann method.

The various experiments conducted may be conveniently grouped under the following headings:

- A. *Tests to prove the sterility of the catgut.*
- B. *Tests to show the effect of catgut on growing cultures.*
- C. *Tests to show the effect of infected catgut.*

A. *Tests to prove the Sterility of the Catgut.*—These experiments were conducted in the following manner. Pieces of iodine catgut and of von Bergmann catgut, about one inch in length, were placed in the following media: Bouillon, gelatin (previously liquefied and poured into a Petri dish), agar-agar (previously liquefied and poured into a Petri dish), and serum-bouillon; the gelatin plates were kept at room-temperature, the remainder in the thermostat, and all were observed from day to day for about two weeks.

Following is an account of these experiments:

EXPERIMENT 1.—December 30. Iodine gut planted into bouillon. December 31, no growth. January 1, no growth. Observations taken until January 14, up to which date there was not the slightest clouding of the medium noted.

EXPERIMENT 2.—December 30. Von Bergmann catgut planted into bouillon. December 31, no growth. January 2, growth, as shown by cloudiness of the medium. Observations taken until January 14, but no further change noted.

EXPERIMENT 3.—December 30. Gelatin was liquefied and poured into a Petri dish; after solidification, a piece of iodine gut was placed upon it. December 31, no growth, nor at any time until January 7, when further observation was discontinued.

EXPERIMENT 4.—December 30. Gelatin was liquefied and poured into a Petri dish; after solidification, a piece of von Bergmann catgut was placed upon it. December 31, no growth. January 2, no growth. Jan-

uary 3, one colony at great distance from the catgut. (Accidental contamination.) Observations taken until January 7, but no further change noted.

EXPERIMENT 5.—Agar-agar was liquefied and poured into a Petri dish; after solidification, a piece of iodine gut was placed upon it. December 31, no growth. January 2, no growth, nor at any time until January 10, when further observation was discontinued.

EXPERIMENT 6.—December 30. Agar-agar was liquefied and poured into a Petri dish; after solidification, a piece of von Bergmann catgut was placed upon it. December 31, no growth. January 2, no growth, nor at any time until January 10, when further observation was discontinued.

EXPERIMENT 7.—December 30. Equal parts of hydrocele-serum and bouillon were mixed, and into this mixture a piece of iodine gut was placed. December 31, no growth, nor at any time until January 14, when further observation was discontinued.

EXPERIMENT 8.—December 30. Equal parts of hydrocele-serum and bouillon were mixed in a test-tube, and into this mixture a piece of von Bergmann catgut was placed. December 31, no growth. Observations taken until January 10, when the medium became turbid, and remained so until January 14, when further observations were discontinued.

EXPERIMENT 9.—December 31. Repetition of Experiment 1; remained sterile until January 14, when further observation was discontinued.

EXPERIMENT 10.—December 31. Repetition of Experiment 2. January 2, no growth. January 3, medium turbid. Observations taken until January 14, but no further change noted.

EXPERIMENT 11.—December 31. Repetition of Experiment 3; remained sterile until January 10, when further observation was discontinued.

EXPERIMENT 12.—December 31. Repetition of Experiment 4. January 2, growth beginning at one end of the catgut, and therefore might be looked upon as a contamination. January 3, the entire plate was overgrown. Observations continued until January 7, but no further change noted.

EXPERIMENT 13.—December 31. Repetition of Experiment 5; remained sterile until January 10, when further observation was discontinued.

EXPERIMENT 14.—December 31. Repetition of Experiment 6; remained sterile until January 10, when further observation was discontinued.

EXPERIMENT 15.—December 31. Repetition of Experiment 7; remained sterile until January 14, when further observation was discontinued.

EXPERIMENT 16.—December 31. Repetition of Experiment 8; remained sterile until January 14, when further observation was discontinued.

It might be argued by some that the iodine catgut, owing to its imbibition with iodine, is so powerful that in a measure it sterilizes the comparatively small amount of bouillon in a test-tube; to refute this argument, I have a number of times repeated the above experiments with iodine gut, but used for each experiment a much larger quantity (60 cubic centimetres) of bouillon, but in no instance did I obtain a growth.

In summing up these sixteen experiments, it is at once evident that all the tests made with the iodine gut remained sterile; some of the tests made with the von Bergmann catgut showed a growth, notably those made with bouillon (Experiments 2 and 10). The von Bergmann catgut is not on trial here, but in view of these tests it certainly shows an inferiority. Suffice it to say that these experiments justify at least the positive conclusion that the iodine catgut is sterile.

B. Tests to show the Effect of the Catgut on growing Cultures.

EXPERIMENT 52.—January 10. A tube of agar-agar was liquefied, infected with *Bacterium coli*, and poured into a Petri dish. After solidification, a piece of iodine catgut was placed upon it. January 11, numberless colonies developed, but none in a space three-quarters of an inch wide surrounding the catgut. January 12, the only change noted was an increase in the size of some of the colonies at the margin of the clear space. Observations taken until January 16, but no further change noted. (See illustration.)*

EXPERIMENT 53.—January 10. A tube of agar-agar was liquefied, infected with *Bacterium coli*, and poured into a Petri dish. After solidification, a piece of von Bergmann catgut was placed upon it. January 11, growth everywhere upon the plate, except in a space about three-sixteenths of an inch in width surrounding the catgut. Observations taken until January 16, but no further change noted. (See illustration.)

EXPERIMENT 54.—January 10. A tube of agar-agar was liquefied, infected with *Staphylococcus aureus*, and poured into a Petri dish. After solidification, a piece of iodine gut was placed upon it. January 11, numberless colonies upon the plate, except in a space about two inches wide surrounding the catgut. Observations taken until January 16, but no material change noted, excepting an increase in the size of the colonies near the margin of the clear space. (See illustration.)

* Dr. Edward Leaming was kind enough to make for me the photographs accompanying this article, for which I wish to express my thanks also on this occasion.

EXPERIMENT 55.—January 10. A tube of agar-agar was liquefied, infected with *Staphylococcus aureus*, and poured into a Petri dish. After solidification, a piece of von Bergmann catgut was placed upon it. January 11, numberless colonies everywhere, excepting in a space about one-eighth of an inch in width surrounding the catgut. Observations taken until January 16, but no further change noted. (See illustration.)

EXPERIMENT 60.—January 10. A tube of gelatin was liquefied, infected with *Bacterium coli*, and poured into a Petri dish. After solidification, a piece of iodine gut was placed upon it. January 11, no growth. January 12, numerous colonies, excepting in a space about two inches wide surrounding the catgut. Observation continued until January 16, but no further change noted.

EXPERIMENT 61.—January 10. A tube of gelatin was liquefied, infected with *Bacterium coli*, and poured into a Petri dish. After solidification, a piece of von Bergmann catgut was placed upon it. January 11, numberless colonies everywhere, excepting in a space about one-quarter of an inch wide surrounding the catgut, and even here a few isolated colonies are to be seen with the microscope. Observations taken until January 16, but no further change noted except an increase in the size of some of the colonies.

EXPERIMENT 62.—January 10. A tube of gelatin was liquefied, infected with *Staphylococcus aureus*, and poured into a Petri dish. After solidification, a piece of iodine catgut was placed upon it. January 11, no growth. January 12, numerous colonies, excepting a space about two inches wide surrounding the catgut. Observations taken until January 16, but no further change noted.

EXPERIMENT 63.—January 10. A tube of gelatin was liquefied, infected with *Staphylococcus aureus*, and poured into a Petri dish. After solidification, a piece of von Bergmann catgut was placed upon it. January 11, no growth. January 12, numerous colonies, except in a space about one-quarter of an inch in width surrounding the catgut. January 13, colonies almost up to the catgut. Observations taken until January 16, but no further change noted.

EXPERIMENT 111.—January 16. A tube of agar-agar was liquefied, infected with *Bacillus anthracis*, and poured into a Petri dish. After solidification, a piece of iodine gut was placed upon it. January 17, numerous colonies, but none within two and one-quarter inches of the catgut. January 18, a large number of isolated colonies developed within the space previously clear, but none within a space of one inch surrounding the catgut. Observations taken until January 21, but no further change noted. (See illustration.)

EXPERIMENT 112.—January 16. A tube of agar-agar was liquefied, infected with *Bacillus anthracis*, and poured into a Petri dish. After solidification, a piece of von Bergmann catgut was placed upon it. January 17, numerous colonies, excepting in a space about one-half inch wide, surrounding the catgut. Observations taken until January 21, but only a slight extension of the colonies noted. (See illustration.)

EXPERIMENT 113.—January 16. A tube of agar-agar was liquefied, infected with *Bacillus anthracis*, and poured into a Petri dish. After solidification, a piece of iodine catgut was placed upon it. January 17, numerous colonies, but none within one and three-quarters inches of the catgut. January 18, a few scattered colonies around the margin of the space previously clear, but still there are no colonies within one inch of the catgut. Observations taken until January 21, but no further change noted, excepting an increase in the size of some of the superficial colonies.

EXPERIMENT 114.—January 16. A tube of agar-agar was liquefied, infected with *Bacillus subtilis*, and poured into a Petri dish. After solidification, a piece of von Bergmann catgut was placed upon it. January 17, numerous colonies, but none within a space, about one-third of an inch wide, surrounding the catgut. Observations taken until January 21, but no further change noted.

EXPERIMENT 91.—January 14. A tube of agar-agar was liquefied, infected with *Streptococcus pyogenes*, and poured into a Petri dish. After solidification, a piece of iodine catgut was placed upon it. January 15, a few colonies to be seen with the microscope, but only at the margins of the Petri dish. Observations taken until January 21, but no further change noted.

EXPERIMENT 92.—January 14. A tube of agar-agar was liquefied, infected with *Streptococcus pyogenes*, and poured into a Petri dish. After solidification, a piece of von Bergmann catgut was placed upon it. January 15, numerous colonies to be seen with the naked eye and microscope, but none in a space about one-half inch wide surrounding the catgut. Observations taken until January 21, but no further change noted.

EXPERIMENT 56.—January 10. Smears of *Bacterium coli* were made upon agar solidified in a Petri dish; upon these a piece of iodine gut was placed. January 11, numerous colonies, but none anywhere near the catgut. January 12, the colonies are larger than on January 11, but there is no extension towards the catgut. Observations taken until January 16, but no further change noted. (See illustration.)

EXPERIMENT 57.—January 10. Smears of *Bacterium coli* were made upon agar solidified in a Petri dish; upon these a piece of von Bergmann catgut was placed. January 11, growth on all smears almost up to the catgut, though not quite in contact with it. Observations taken until January 16, but only an increase in the size of the colonies noted. (See illustration.)

EXPERIMENT 58.—January 10. Smears of *Staphylococcus aureus* were made upon agar solidified in a Petri dish; upon these a piece of iodine gut was placed. January 11, growth on all smears distant from the catgut, but none anywhere near it. Observations taken until January 16, but only an increase in the size of the colonies noted. (See illustration.)

EXPERIMENT 59.—January 10. Smears of *Staphylococcus aureus* were made upon agar solidified in a Petri dish; upon these a piece of von Bergmann catgut was placed. January 11, growth on all smears, almost up to the catgut, but not quite in contact with it. January 12, increase in the size of the colonies, and their extension towards the catgut was

noted, so as to be almost in contact with it. Observations taken until January 16, but only an increase in the size of the colonies noted. (See illustration.)

EXPERIMENT 66.—January 11 is a repetition of Experiment 56, and is inserted here merely on account of the perfect illustration, which see.

EXPERIMENT 67.—January 11 is a repetition of Experiment 57, and is inserted here merely on account of the perfect illustration, which see.

EXPERIMENT 68.—January 11 is a repetition of Experiment 58, and is inserted here merely on account of the perfect illustration, which see.

EXPERIMENT 69.—January 11 is a repetition of Experiment 59, and is inserted here merely on account of the perfect illustration, which see.

EXPERIMENT 93.—January 14. Smears of *Bacillus anthracis* were made upon agar solidified in a Petri dish; upon these a piece of iodine gut was placed. January 15, a few colonies developed near the ends of the streaks, but none anywhere near the catgut. Observations taken until January 21, but no further change noted. (See illustration. The colonies to the left of the catgut in this illustration are not of anthrax, but are accidental contaminations, developing sometime after the experiment.)

EXPERIMENT 94.—January 14. Smears of *Bacillus anthracis* were made upon agar solidified in a Petri dish; upon these a piece of von Bergmann catgut was placed. January 15, abundant growth on all streaks up to one-quarter of an inch from the catgut. Observations taken until January 21, but only an increase in the size of the colonies noted. (See illustration.)

EXPERIMENT 95.—January 14. Smears of *Bacillus subtilis* were made upon agar solidified in a Petri dish; upon these a piece of iodine gut was placed. January 15, growth at both ends of the streaks, but none anywhere near the catgut. Observations taken until January 21, but no further change noted. (See illustration.)

EXPERIMENT 96.—January 14. Smears of *Bacillus subtilis* were made upon agar solidified in a Petri dish; upon these a piece of von Bergmann catgut was placed. January 15, plate overgrown with extensive colonies in actual contact with the catgut. Observations taken until January 21, but no further change noted. (See illustration.)

EXPERIMENT 97.—January 14. Smears of *Streptococcus pyogenes* were made upon agar solidified in a Petri dish; upon these a piece of iodine gut was placed. January 15, a few colonies developed towards the ends of some of the streaks, all far away from the catgut. Observations taken until January 21, but no further change noted.

EXPERIMENT 98.—January 14. Smears of *Streptococcus pyogenes* were made upon agar solidified in a Petri dish; upon these a piece of von Bergmann catgut was placed. January 15, numerous colonies developed, but none nearer than one-half inch from the catgut. Observations taken until January 21, but no further change noted.

Even a hasty examination of the experiments just related, and particularly of the accompanying photographs, at once calls

attention to two phenomena; first, the relatively large area, upon the plates made with iodine gut, which is free from any growth, more particularly when compared with the control plates made with von Bergmann catgut. It is, of course, difficult to express exactly in figures how much larger the area is around the iodine catgut than around the von Bergmann catgut; but I believe myself to be within safe limits if I judge that it is at least four times as great. Secondly, though relatively small, there is still an area free from growth also surrounding the von Bergmann catgut. It is self-evident that the large area surrounding the iodine catgut is due to the powerful antiseptic properties of the free iodine contained therein; and it was but natural to assume some similar agent, *e.g.*, HgCl_2 , was also present in the von Bergmann catgut. Dr. Bookman, physiological chemist of Mount Sinai Hospital, was kind enough to undertake for me a chemical analysis. He found that one yard of No. 1 catgut prepared by the von Bergmann method contained an amount of mercury which would be equivalent to 0.008825 of HgCl_2 ; this will readily explain the relatively small sterile area upon the plates.

I am particularly pleased to acknowledge this work of Dr. Bookman, as at first I was at a loss to account for the non-appearance of any growth in the plates made with the von Bergmann catgut. I personally was always under the impression that in the von Bergmann catgut we are dealing with an aseptic catgut, and not with an antiseptic catgut; but the results obtained in the chemical analysis prove the contrary. It appears that the bichloride of mercury used in the preparation of the catgut enters into a chemical combination with the organic substances, probably some form of albuminate, which evidently has antiseptic properties as well.

It is patent to everybody that the sterility of the iodine catgut is due to the antiseptic action of the iodine contained therein; but it was of interest to me, also, to know just how much iodine was contained in a given piece of iodine catgut. Analysis by Dr. Bookman revealed the presence of 0.025737 of iodine in one yard of No. 1 iodine catgut. If it is recollected

that a 1 to 5000 aqueous solution of iodine is still a very powerful antiseptic, it will be seen that one and one-half inches of No. 1 catgut will be amply sufficient to render absolutely sterile 8 cubic centimetres of medium, the amount generally used for bacteriological purposes.

It might, and with a certain amount of justice, be argued that in the experiments just related, the iodine exerts merely an inhibitory action upon the development of the bacteria, and that there are still numerous active and living bacteria in the clear area surrounding the catgut, which are only temporarily prevented from developing, but which would still develop when placed in more favorable surroundings. In order to disprove such a possibility, I have made the following experiments:

EXPERIMENT A. EXPERIMENT 162.—January 29. A tube of liquefied agar-agar was inoculated with a virulent culture of *Bacillus anthracis*, and poured into a Petri dish. After solidification, a piece of iodine catgut was placed upon it. January 30, numerous colonies developed, but none in an area extending over one inch on either side of the catgut. February 1, no further change noted.

EXPERIMENT 164.—January 30. A liberal inoculation was made from the clear agar surrounding the iodine catgut in Experiment 162, and about one-half inch away from it, into bouillon. January 31, no growth. Observations taken until February 5, but no further change noted.

EXPERIMENT 166.—January 30. Repetition of Experiment 164 in agar and Petri dish also gave a negative result.

But even this series of experiments may not be considered absolutely conclusive, as it might be argued that the conditions with the bouillon in Experiment 164, or with the agar in Experiment 166, were still not sufficiently favorable for the development of the anthrax bacilli. I therefore supplemented them with the following animal experiment.

EXPERIMENT 172.—February 4. After proper preparation and antiseptic precautions, a small incision was made upon the back of a guinea-pig, and with dressing-forceps a small pocket was prepared. Into this there was buried a piece of the clear agar surrounding the catgut in Experiment 162, about one-half inch square and about one-sixteenth of an inch thick. February 5, no reaction. February 11, animal still alive and perfectly well.

Surely in this test all the conditions favorable for the development of any bacteria were present; and if the high virulence of the particular anthrax culture used be considered, we must assume that death of the animal should have followed, provided there were still living, though undeveloped, bacteria in the clear space surrounding the catgut.

In corroboration of this fact, I might also adduce the argument that all of my plates were observed for a number of days in the thermostat, and that I have found that already after twenty-four hours, owing to the temperature of the thermostat, and owing to the chemical affinity free iodine has for the various salts contained in the nutritive media, the catgut has lost its black color, nor could I get the well-known reaction for iodine with starch. It follows therefore that any agency which the iodine may have upon the non-development of bacteria must be exerted in the beginning; assuming merely an inhibitory action, this would certainly cease after the iodine has been driven off by volatilization; but as no bacteria develop in the clear space even after long-continued observation, we must come to the conclusion that the iodine acts not merely as an inhibitory agent, but as a true bactericide.

I know full well that the human body is not a culture tube nor a guinea-pig, and that numerous other conditions, which exist in the human body and not in the Petri dish, must be taken into account; but I believe we may safely deduct this much from this series of experiments, namely, that the iodine catgut will, at least in a measure, assist in neutralizing accidental infections in wounds.

C. Tests to show the Effects of Infected Catgut.—I now come to the most interesting series of my experiments,—interesting because they were entirely unlooked for and surprising, and also because they are so very important from the surgeon's standpoint. This series of experiments goes to prove in a bacteriological sense that the iodine catgut is practically non-infectible. The importance of this point will be readily appreciated when it is remembered that accidental contact-infections of the catgut are so manifoldly possible, that a careful super-

vision of any operation goes to show that these accidental contact-infections, in spite of all care to eliminate them, are not only not exceptional, but the rule. The surgeon's and assistants' hands, as well as the skin of the patient, can never be rendered absolutely sterile, but only relatively so. The surgeon and assistants may even avoid direct contact with the catgut by wearing rubber gloves, but the skin of the patient cannot be so protected, and yet the catgut, if used as a suture, must pass through it. This results, as we all know, in an occasional stitch abscess, and from time to time in more serious infections. I venture to say that even these may be eliminated with the aid of iodine catgut.

The method of procedure was the following: Bouillon cultures were made of the following actively growing bacteria: *Bacillus coli*; *Staphylococcus aureus*; *Streptococcus pyogenes*; *Bacillus anthracis*, and *Bacillus subtilis*. This culture was kept in the thermostat for twenty-four hours. At the expiration of this time, when an abundant growth had resulted, the culture was divided into two halves, and poured into sterile Petri dishes; into one dish there were placed about one dozen pieces of iodine catgut, about one inch in length, and into the other a similar number of von Bergmann catgut of the same length. Both dishes were then replaced for another twenty-four hours into the thermostat. At this time the excess of culture medium was poured off, and various tests made with the catgut (in the experiments this is called "wet catgut"); the balance was replaced for another twenty-four hours into the thermostat, and another set of experiments was made with it (in the experiments this is called "dry catgut").

EXPERIMENT 43.—January 5. Iodine gut infected with *Bacillus coli* "wet" planted into bouillon. January 6, no growth, nor at any time until January 14, when further observation was discontinued.

EXPERIMENT 44.—January 5. Von Bergmann catgut infected with *Bacillus coli* "wet" planted into bouillon. January 6, growth. Observations taken until January 14, but no further change noted.

EXPERIMENT 45.—January 5. Iodine gut infected with *Staphylococcus aureus* "wet" planted into bouillon. January 6, no growth, nor at any time until January 14, when further observation was discontinued.

EXPERIMENT 46.—January 5. Von Bergmann catgut infected with *Staphylococcus aureus* "wet" planted in bouillon. January 6, growth. Observations taken until January 14, but no further change noted.

EXPERIMENT 115.—January 17. Iodine gut infected with *Bacillus anthracis* "wet" planted in bouillon. January 18, no growth, nor at any time until January 26, when further observation was discontinued.

EXPERIMENT 116.—January 17. Von Bergmann catgut infected with *Bacillus anthracis* "wet" planted into bouillon. January 18, no growth. January 19, growth. Observations taken until January 26, but no further change noted.

EXPERIMENT 117.—January 17. Iodine gut infected with *Bacillus subtilis* "wet" planted in bouillon. January 18, no growth, nor at any time until January 26, when further observation was discontinued.

EXPERIMENT 118.—January 17. Von Bergmann catgut infected with *Bacillus subtilis* "wet" planted in bouillon. January 18, growth. Observations taken until January 26, but no further change noted.

EXPERIMENT 119.—January 17. Iodine gut infected with *Streptococcus pyogenes* "wet" planted in bouillon. January 18, no growth, nor at any time until January 26, when further observation was discontinued.

EXPERIMENT 120.—January 17. Von Bergmann catgut infected with *Streptococcus pyogenes* "wet" planted in bouillon. January 18, growth. Observations taken until January 26, but no further change noted.

These ten experiments made with the two varieties of catgut, after having been literally soaked for twenty-four hours in a twenty-four-hour old culture of actively growing bacteria, show that the iodine content of the iodine catgut was sufficient at least to inhibit, if not destroy, the development of bacteria with which it came in contact, since all the experiments with the iodine catgut gave a negative result, while all the experiments with the von Bergmann catgut gave a positive result. As in actual practice, however, we are using the catgut dry, it was necessary to repeat all these experiments, also, with the dried catgut. Following were the results obtained:

EXPERIMENT 47.—January 6. Iodine gut infected with *Bacillus coli* "dry" planted in bouillon. January 7, no growth, nor at any time until January 14, when further observation was discontinued.

EXPERIMENT 48.—January 6. Von Bergmann catgut infected with *Bacillus coli* "dry" planted in bouillon. January 7, growth. Observations taken until January 14, but no further change noted.

EXPERIMENT 49.—January 6. Iodine gut infected with *Staphylococcus aureus* "dry" planted in bouillon. January 7, no growth, nor at any time until January 14, when further observation was discontinued.

EXPERIMENT 50.—January 6. Von Bergmann catgut infected with *Staphylococcus aureus* "dry" planted in bouillon. January 7, no growth. January 8, growth. Observations taken until January 14, but no further change noted.

EXPERIMENT 121.—January 18. Iodine gut infected with *Bacillus anthracis* "dry" planted in bouillon. January 19, no growth, nor at any time until January 26, when further observation was discontinued.

EXPERIMENT 122.—January 18. Von Bergmann catgut infected with *Bacillus anthracis* "dry" planted in bouillon. January 19, no growth. January 20, no growth. Observations taken until January 26, but no further change noted. (The only negative experiment in the entire series!)

EXPERIMENT 123.—January 18. Iodine gut infected with *Bacillus subtilis* "dry" planted in bouillon. January 19, no growth, nor at any time until January 26, when further observation was discontinued.

EXPERIMENT 124.—January 18. Von Bergmann catgut infected with *Bacillus subtilis* "dry" planted in bouillon. January 19, growth. Observations taken until January 26, but no further change noted.

EXPERIMENT 125.—January 18. Iodine catgut infected with *Streptococcus pyogenes* "dry" planted in bouillon. January 19, no growth, nor at any time until January 26, when further observation was discontinued.

EXPERIMENT 126.—January 18. Von Bergmann catgut infected with *Streptococcus pyogenes* "dry" planted in bouillon. January 19, growth. Observations taken until January 26, but no further change noted.

I have repeated the experiments just related also with agar media; their object is merely to serve as a better object lesson. The procedure was the following: Agar-agar was liquefied and poured into Petri dishes. After solidification, a piece of the dry catgut, infected according to the method before described, was placed upon it, and the subsequent behavior noted. Following was the result of these experiments:

EXPERIMENT 72.—January 12. Iodine gut infected with *Bacillus coli* "dry" placed upon agar. January 13, no growth, nor at any time until January 17, when further observation was discontinued. (See illustration.)

EXPERIMENT 73.—January 12. Von Bergmann catgut infected with *Bacillus coli* "dry" placed upon agar. January 13, growth. Observations taken until January 17, but no further change noted. (See illustration.)

EXPERIMENT 74.—January 12. Iodine gut infected with *Staphylococcus aureus* "dry" placed upon agar. January 13, no growth, nor at any time until January 17, when further observation was discontinued. (See illustration.)

EXPERIMENT 75.—January 12. Von Bergmann catgut infected with *Staphylococcus aureus* "dry" placed upon agar. January 13, growth. Observations taken until January 17, but no further change noted. (See illustration.) (In this illustration the large colony at the upper end is an accidental mould, but the small, fine colonies along the lower end are typical staphylococci.)

EXPERIMENT 127.—January 18. Iodine gut infected with *Bacillus anthracis* "dry" placed upon agar. January 19, no growth, nor at any time until January 26, when further observation was discontinued. (See illustration. No attention is to be paid to the accidental contamination to the right of this illustration, nor in the next illustration.)

EXPERIMENT 128.—January 18. Von Bergmann catgut infected with *Bacillus anthracis* "dry" placed upon agar. January 19, no growth. January 20, growth. Observations taken until January 26, but no further change noted. (See illustration.)

EXPERIMENT 129.—January 18. Iodine gut infected with *Bacillus subtilis* "dry" placed upon agar. January 19, no growth, nor at any time until January 26, when further observation was discontinued. (See illustration.)

EXPERIMENT 130.—January 18. Von Bergmann catgut infected with *Bacillus subtilis* "dry" placed upon agar. January 19, growth. Observations taken until January 26, but no further change noted. (See illustration.)

EXPERIMENT 131.—January 18. Iodine gut infected with *Streptococcus pyogenes* "dry" placed upon agar. January 19, no growth, nor at any time until January 26, when further observation was discontinued.

EXPERIMENT 132.—January 18. Von Bergmann catgut infected with *Streptococcus pyogenes* "dry" placed upon agar. January 19, no growth. January 20, growth. Observations taken until January 26, but no further change noted.

The thirty experiments last enumerated are of the utmost importance, because they more nearly approach the use of catgut in actual practice. They show that at least, as far as the bacteriological test is concerned, the iodine catgut is far superior to the von Bergmann catgut, inasmuch as it is absolutely "uninfectible." All experiments with the iodine catgut gave an absolutely negative result, while with one exception (Experiment 122) all experiments made with the von Bergmann catgut gave a positive result. Again, I would call attention to the fact that the living human organism is not a culture tube, and it would be wrong to transfer inferences from these test-tube and Petri dish experiments to the operating room; but they certainly show this at least, that those constantly occur-

ring, accidental, and unavoidable infections are of no harm if they occur with iodine catgut, while similar infections of the von Bergmann catgut may be productive of the greatest harm.

In addition to the above experiments, I have also made a few experiments with iodine gut after infecting it with pus from various sources. Of course, these experiments lose in actual value when compared with those just related, in which we deal with certain definite and positive infections; but they appeal perhaps more to the surgeon, as they deal with infections which might occur at any time in an operating room, or even in the course of an individual operation.

EXPERIMENT 51.—January 10. On January 9 I had occasion to operate a metastatic empyema, caused by streptococci, the presence of which was verified both by slides and cultures. A basin of the pus was caught in a sterile vessel, and in it there was placed a spool of iodine catgut. After an immersion of six hours it was removed and placed into a sterile bottle, with large amounts of the pus still adhering to it. Fourteen hours later (*i.e.*, twenty hours after beginning the experiment) a piece of the catgut about one inch in length was planted into bouillon. January 11, no growth, nor at any time until January 16, when further observation was discontinued.

To refute the possible argument that in this as in other experiments the amount of iodine contained in the catgut was sufficient to render the small quantity of bouillon antiseptic, the following experiment was made:

EXPERIMENT 64.—January 11. A piece of the catgut used in Experiment 51, about one inch in length, was planted into a large quantity (60 cubic centimetres) of bouillon, forty hours after the empyema operation. January 12, no growth, nor at any time until January 18, when further observation was discontinued.

EXPERIMENT 70.—January 12. Iodine catgut was liberally smeared in the pus of a freshly opened furuncle of the neck (*staphylococcus* in spreads), and one hour later a piece one inch in length was planted into bouillon. January 13, no growth, nor at any time until January 18, when further observation was discontinued.

EXPERIMENT 71.—January 12. Iodine catgut was liberally smeared in the pus of an axillary abscess (*staphylococci* in spreads), and one hour later a piece one inch in length was planted into bouillon. January 13, no growth, nor at any time until January 18, when further observation was discontinued.

EXPERIMENT 76.—January 13. The iodine gut used in Experiment 70 was allowed to remain in contact with the pus, and twenty-six hours later, by which time it had become perfectly dry, a piece one inch in length was planted into bouillon. January 14, no growth, nor at any time until January 21, when further observation was discontinued.

EXPERIMENT 77.—January 13. The iodine gut used in Experiment 71 was allowed to remain in contact with the pus, and twenty-six hours later, by which time it had become perfectly dry, a piece one inch in length was planted into bouillon. January 14, no growth, nor at any time until January 21, when further observation was discontinued.

EXPERIMENT 78.—January 13. Iodine gut was smeared in the pus of a freshly incised large periproctitic abscess, communicating by a fistulous tract with the rectum (numerous cocci and bacilli in spreads), and one hour later a piece one inch in length was planted into bouillon. January 14, no growth, nor at any time until January 21, when further observation was discontinued.

EXPERIMENT 80.—January 14. The iodine gut used in Experiment 78 was allowed to remain in contact with the pus for twenty-five hours, by which time it had become perfectly dry, and a piece one inch in length was planted into bouillon. January 15, no growth, nor at any time until January 21, when further observation was discontinued.

EXPERIMENT 82.—January 14. Iodine gut was smeared into the pus of a freshly incised suppurative arthritis of the elbow-joint, and one hour later a piece one inch in length was planted into bouillon. January 15, no growth, nor at any time until January 21, when further observation was discontinued.

On this occasion I repeated the experiment also with von Bergmann catgut, and, as was to be expected, obtained a positive result.

EXPERIMENT 83.—January 14. Von Bergmann catgut was smeared into the pus of a freshly incised suppurative arthritis of the elbow-joint, and one hour later a piece one inch in length was planted into bouillon. January 15, growth.

In addition, I have made numerous experiments in the following manner: Pieces one inch in length were cut with an ordinary old unsterilized pair of scissors, from spools of iodine catgut, which had lain some time for months in a not overclean laboratory drawer, and planted into bouillon, but at no time was a growth obtained.

All of the experiments just related show that at least, as far as culture experiments go, not only is the iodine catgut absolutely sterile, but also that it is absolutely impossible to infect it, either with cultures of known activity or with resistant spores, or with ordinary pus.

Advisedly and with caution have I used in my *résumé* the expression that these results are true as far as the culture experiments are concerned, as I expected that the objection would be raised that in an operation we would have to deal with conditions which materially differ from any laboratory experiment. This was brought to my attention by Dr. Lilienthal, of Mount Sinai Hospital, who conceded that the catgut was sterile, and also that it was uninfectible, provided sufficient time was given for the iodine to exert its antiseptic action. He argued that in the course of an operation infections of the catgut might occur, which catgut being used immediately thereafter, before the iodine had sufficient time to exert its bactericidal action, might possibly cause an infection in this manner. Dr. Lilienthal was of the opinion that a nearer approach to the actual use of the catgut could be obtained in the following manner: That catgut infected with various germs is inoculated into a nutritive medium, but is removed therefrom in a few seconds. While conceding the validity of the argument, I would not concede the validity of the experiment, but, *experimenti causa*, I have carried out the tests suggested, using both iodine and von Bergmann catgut; the result of which in brief is the following:

EXPERIMENT 133.—January 19. Immersion for a few seconds of iodine gut infected with *Bacillus coli* into bouillon was followed by no growth.

EXPERIMENT 134.—January 19. Immersion for a few seconds of von Bergmann catgut infected with *Bacillus coli* into bouillon was followed by a growth.

EXPERIMENT 135.—January 19. Immersion for a few seconds of iodine gut infected with *Staphylococcus aureus* into bouillon was followed by no growth.

EXPERIMENT 136.—January 19. Immersion for a few seconds of von Bergmann catgut infected with *staphylococcus aureus* into bouillon was followed by no growth.

EXPERIMENT 137.—January 19. Immersion for a few seconds of iodine gut infected with *Bacillus anthracis* into bouillon was followed by no growth.

EXPERIMENT 138.—January 19. Immersion for a few seconds of von Bergmann catgut infected with *Bacillus anthracis* into bouillon was followed by no growth.

EXPERIMENT 139.—January 19. Immersion for a few seconds of iodine gut infected with *Bacillus subtilis* into bouillon was followed by no growth.

EXPERIMENT 140.—January 19. Immersion for a few seconds of von Bergmann catgut infected with *Bacillus subtilis* into bouillon was followed by a growth.

While, as already stated, I do not concede the validity of the argument that the experiments just related are a closer approach to infections as they might occur accidentally in the course of an operation, they are of interest in so far that they also tend to prove that it is impossible to get infections with the iodine catgut in this manner; all the experiments with iodine catgut gave a negative result, while on the other hand two out of the five experiments with the von Bergmann gut (the coli and subtilis experiment) gave a positive result.

In order to determine the possibility of such infections, experiments would have to be done actually upon the human body; but, as this would be entirely too risky and dangerous, no one will reproach me with the incompleteness of my studies. I have attempted to approach this form of infection with animal experiments, and following is the result:

EXPERIMENT 148.—January 27. On January 26 I made a bouillon culture of an actively growing, virulent *Bacillus anthracis*, and permitted it to grow for twenty-four hours in the thermostat. On January 27 I made a small incision upon the back of a guinea-pig, and bluntly made therein a subcutaneous channel; into this I buried two pieces of iodine catgut about two inches in length, which had been dipped into the anthrax culture, and closed the opening with a suture. January 28. The animal looked and acted perfectly well, and, in spite of this extremely severe test, I had strong hopes that the animal might overcome the infection. In this I was disappointed, as on the following morning, January 29, the animal was found dead.

EXPERIMENT 147.—January 27. In this experiment I repeated in all details Experiment 148, with the exception that I used von Bergmann catgut. On January 28 the animal looked and acted sick and refused its nourishment. On the following morning, January 29, the animal was found dead.

It is seen, therefore, that both these experiments (148 and 147) gave a negative result, inasmuch that both animals died. Nor could I expect anything different; and I so ex-

pressed myself at the time I operated upon the animals. I know that sufficient of the culture adhered to the dripping wet catgut to kill any animal, and I also know that in the introduction of the catgut sufficient of the culture medium was wiped upon the wound at some distance from the iodine catgut to cause an infection and death. The only conclusion to be reached, therefore, is that both animals died in consequence of a surcharge of the infective medium; and I am sure every one will agree with me that this is not the way catgut infections occur in the course of an operation, even if we would concede the possibility of anthrax infection.

It was necessary, therefore, to find a method which would more nearly approach the conditions as they might occur in practice. I believe I have found it by proceeding in the following manner:

EXPERIMENT 170.—February 4. Two pieces of iodine gut, about two inches in length, were thoroughly moistened in a twenty-four-hour bouillon culture of virulent anthrax; they were then dried between folds of sterile blotting-paper, and immediately buried in a channel beneath the skin of the back of a guinea-pig. February 5, the animal looks and acts perfectly well. February 6, apparently no change in the condition and behavior of the animal. February 7, the animal was found dead in its cage.

EXPERIMENT 171.—February 4. Repetition of Experiment 170, with the difference that in place of iodine catgut von Bergmann catgut was used. February 5, the animal was found dead in its cage.

It is seen that the last two experiments (170 and 171) were followed by the same result as the more severe experiments (147 and 148); both animals, the one with infected iodine gut as well as the one with infected von Bergmann catgut, died. It is true that the former outlived the latter by forty-eight hours, but that is, and should be, no criterion. What we are looking for are positive results in the form of complete immunity, and not merely prolongation of life; at best, we are entitled perhaps merely to deduct so much from these experiments that the iodine catgut was sufficiently strong to neutralize some of the anthrax bacilli, but there still remained sufficient to eventually kill the guinea-pig. Even this,

however, would be some encouragement from the surgeon's point of view, as it must be recollected that, as a general rule, we are not working with catgut which has been soaked, like in the experiments related, in virulent anthrax cultures.

All in all, I do not see how we could imitate the conditions exactly to fulfil the objections raised by Dr. Lilienthal. It is true that I could perhaps repeat the experiments with bacteria, which are less fatal than anthrax, for instance, with the ordinary pus-forming germs, but I do not see that much would be gained by it. For instance, the presence of pus would by no means be proof of the inefficiency of the iodine catgut, as it might be argued that the purulent infection was introduced otherwise and not with the catgut. I confess, therefore, that, for the want of a good method, I am for the present unable to refute Dr. Lilienthal's argument.

The result of the various experiments carried out to prove the sterility of the catgut are to my mind so convincing that a general *résumé* is hardly indicated.

2. TENSILE STRENGTH.—Having proven the aseptic and antiseptic properties of iodine catgut, it remained for me to show that its tensile strength was not inferior, and perhaps superior, to other forms of catgut.

It is not sufficient to state that in actual use we have found that the catgut was sufficiently strong for all the requirements, or all the demands made upon it; nor can much value be put upon the statements of others that the catgut was too weak, and that it has lost a considerable part of its tensile strength. A great deal depends upon the personal equation of the individual who is using or testing it. If "A" wishes to prove that any catgut is strong, he does not pull quite as hard as "B," who wishes to prove that the catgut is weak, and *vice versa*. The only way to either prove or disprove anything of this nature is by actually and accurately measuring the amount of pulling force applied. This would be comparatively easy with most substances, but with catgut it is surrounded by almost insurmountable difficulties. This is due to many facts about

catgut, all of which have a most important bearing upon its tensile strength. First, there exist no accurate standard sizes of catgut. It is true that we can buy in the market so-called standard sizes, which are variously labelled from 00 upward; but in reality this does not mean anything, as anybody can readily convince himself by simply glancing at a number of rolls. I have, for instance, frequently examined packages of catgut marked for a certain size, and found all possible variations, both above and below the size stated; in some of the larger sizes measured (No. 2) with an accurate micrometer the variations were as much as fifteen one-hundredths of a millimetre in diameter. Fifteen one-hundredths of a millimetre may appear a triviality, but in reality it makes a very great difference, as the strength of a given piece of material varies not as does the diameter, but as does the square of the diameter. Second, there is an important variation in size, not only between different rolls, but also in different parts of the same roll, and we find thinner portions alternating with thicker portions; and it is perhaps needless to add that a given piece of the catgut is only as strong as its weakest portion. Third, that the strength of the catgut depends not only on its diameter, but also upon other undetermined qualities, which vary not only in different rolls, but also in different portions of the same roll, *e.g.*, light portions alternate with dark portions, translucent with almost transparent portions, alternate with opaque portions. Finally, that the strength of the catgut depends upon the workmanship originally used in its preparation; I mean how tightly it was originally twisted to get a diameter of a certain size. It is self-evident that of two pieces of catgut of equal diameter that one will be stronger which is twisted tighter, because in reality there is much more catgut substance in it. I have paid a good deal of attention to this point, and finally, by a mere glance, I could tell beforehand which catgut would be strong and which weak. All in all, it is seen that this was by no means an easy question to solve.

But after making due allowance for these qualities inherent to the catgut, there arose a further difficulty, inasmuch

as I was not in possession of any apparatus by which I could accurately determine its tensile strength. The crude affairs that I was able to rig up were not sufficiently accurate, and

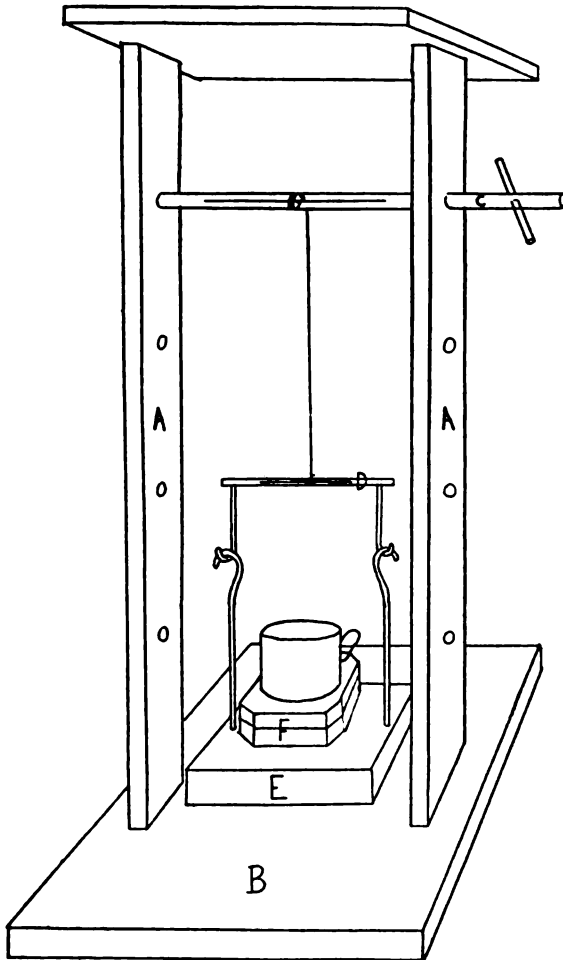


FIG. 1.—Apparatus to test tensile strength of catgut.

were so cumbersome that I could not make sufficient headway. In this dilemma I consulted Professor Hallock, of the Department of Physics, at Columbia University; as no apparatus was

on hand to determine the tensile strength of catgut, Dr. Forbes, of the Department of Physics, of Columbia University, very kindly designed and constructed the following apparatus for me (Fig. 1).

The instrument consists of two parallel vertical bars (A, A) firmly fixed in a stand (B) and connected above. The sides of A and A are perforated at different heights, and through any pair of these holes a cross-bar (C) made of brass may be firmly fixed; the latter is perforated by a long slit and fine openings, through which the catgut may easily be threaded; by a few rotations of C the catgut will be held solidly, and yet will hang only in a tangent from the round surface of C, so as not to be cut by any sharp edge. The other end of the catgut is then fixed in a similar manner to another brass bar (D) which supports a suspended platform (E). This platform in turn supports any number of weights (F) which may be placed upon it. On top of the weights there rests a small cup into which shot may be poured. When the amount of shot poured in is sufficient to break the catgut, it is weighed and the amount added to the original weights first placed upon the platform, plus the weight of the other suspended parts of the apparatus, which, of course, have previously been determined.

While this apparatus is by no means perfect, we believe it to be more devoid of gross errors than apparatus which work with a spring-balance, as it enables us to get nearer to the actual breaking strength of an individual piece of catgut. Soon after the construction of the apparatus, however, I found that a great deal depended on the intrinsic characteristics of the catgut, and that it would not do to merely measure a single piece of the catgut of a given size, and from it to draw the conclusion that this size of catgut has a given breaking-point. The figures vary so much, that the only way to determine this was to determine the breaking-point of many rolls, and from different parts of the same roll, and then to draw the final deduction from the grand average.

Following is the result of my measurements:

NUMBER 0 CATGUT.

Iodine Catgut. Grammes.	Von Bergmann Catgut. Grammes.	Raw Catgut. Grammes.
2827	3595	2801
3778	3691	3226
2616	2731	3216
4241	3136	
3791	3013	
3576	2912	
3696	3078	
4182	3126	
4017	2987	
3928	3175	
—	—	—
Average, 3665	3180	3081

NUMBER 1 CATGUT.

Iodine Catgut. Grammes.	Von Bergmann Catgut. Grammes.	Raw Catgut. Grammes.
4723	4681	4561
4067	3866	4866
4916	4421	5456
6186	4567	
5796	4658	
4911	4649	
5941	5018	
6076	4925	
5866	4532	
5978	4612	
—	—	—
Average, 5446	4592	4961

NUMBER 2 CATGUT.

Iodine Catgut. Grammes.	Von Bergmann Catgut. Grammes.	Raw Catgut. Grammes.
7150	7291	6896
7251	7520	6686
6526	7440	6496
7080	6975	
8716	6942	
6826	7257	
7043	7261	
7251	7223	
7120	6711	
8239	6709	
—	—	—
Average, 7320	7132	6526

While this gives us an excellent and very fair idea of the tensile strength of the catgut as it is used for suture material, it is by no means the tensile strength of the catgut as it is used for ligatures, particularly for tying heavy pedicles; because in the latter we have to deal, in addition, with the knot problem, which was found to be a most important element in the tensile strength of catgut. It was necessary, therefore, to repeat all of these experiments with the addition of a knot in the catgut measured; for the sake of simplicity only a single knot was tied; no doubt a surgeon's knot would give still greater differences.

Following was the result of these measurements:

NUMBER 0 CATGUT.		
Iodine Catgut. Grammes.	Von Bergmann Catgut. Grammes.	Raw Catgut. Grammes.
2146	Less than 1000 *	2076
2151	Less than 1000 *	2021
2166	Less than 1000 *	1856
2351	2346	
2306	2161	
2243	2246	
2116	2361	
2296	2111	
—	—	—
Av'age, 2220	2244	1984

NUMBER 1 CATGUT.		
Iodine Catgut. Grammes.	Von Bergmann Catgut. Grammes.	Raw Catgut. Grammes.
2821	3068	3230
2786	3148	2871
2655	4106	2886
3783	2900	
3113	3801	
3491	3946	
4321	2995	
4196	3161	
4229	4120	
4176	3221	
—	—	—
Av'age, 3557	3446	2996

* Not figured in the average.

NUMBER 2 CATGUT.		
Iodine Catgut. Grammes.	Von Bergmann Catgut. Grammes.	Raw Catgut. Grammes.
4431	5636	3961
4885	5541	3513
4011	5840	3928
4138	4005	
4126	5311	
4081	5124	
4951	5146	
4306	5634	
5356	5214	
5606	5139	
5066	5071	
5184	4923	
—	—	—
Av'age, 4678	5217	3800

The tests with the unknotted catgut, in spite of the great variability in strength, show that the raw catgut is by far the weakest of the three, and that the iodine gut is by far the strongest; this is particularly the case with the smaller sizes, the size most frequently used both as suture and ligature material.

If we now turn our attention to the knotted catgut, we again find that the raw catgut is by far the weakest; Numbers 0 and 1 have practically the same tensile strength if prepared with iodine as with the von Bergmann method, though even here there is some advantage in favor of the iodine catgut; but with the Number 2 catgut there is evidently some advantage in favor of the von Bergmann catgut. I believe, however, that this is merely accidental, as in some spools the iodine catgut gave just as high values as the best of the von Bergmann catgut. It is not impossible that more extensive measurements will equalize these values.

All in all, I am perfectly satisfied that the iodine catgut has not only not lost any of its tensile strength, but apparently it has materially gained.

3. METHOD OF PREPARATION.—Under this heading I can only repeat what I have already stated in my article previously referred to, viz., "The preparation of this catgut is simplicity

itself." But, for the sake of completeness and because of a slight modification in its preservation, it may perhaps not be amiss if I again go into detail regarding this point. The catgut, just as it is bought from the dealers, *i.e.*, without removing the fat, is loosely wound, preferably in a single layer, on to the spool, and tied at both ends in order to prevent unravelling. It is then immersed for eight days in a solution of iodine, one part; iodide of potassium, one part; distilled water, one hundred parts. (The solution is prepared by dissolving the iodide of potassium in a small quantity of the water, to which the iodine, previously finely pulverized, is added, and the whole diluted up to one hundred parts.)

According to Claudius's directions, the catgut was preserved in the original solution without any change; but as already stated, having found that after long-continued immersion in the solution the catgut loses some of its tensile strength, it was necessary to obviate or circumvent this drawback. This was done in a very simple manner, inasmuch as all that was necessary was to remove the catgut from the solution at the end of the eight days, and to preserve it thereafter merely dry in a sterile vessel, preferably in one not exposed to the light. (If necessary, the drying process might be hastened by placing to one side of the vessel in which the catgut is kept a small vial containing a little sulphuric acid.) In short, formerly we used a catgut which was wet, and by the newer method we use it dry.

It is used dry just as it is cut from the spool, without any previous immersion in carbolic solution or sterile water. Any unused catgut may be resterilized on a future occasion.

That this method of preparation is simple no one can deny; that it is extremely cheap, itself no mean item, particularly in large hospitals, where quantities are used, is also self-evident; one gallon of the solution, requiring 608 grains of iodine and an equal amount of iodide of potassium, costing only forty-two cents.

It is important to keep the solution in well-stoppered bottles or jars because the iodine is volatile, as can be seen by

the purple color of the atmosphere on top of the solution, and in time it deteriorates. Solutions good enough to use should in bulk have a deep brown, almost black color; any solution not corresponding to these physical requirements should be discarded.

The new method of preservation has produced also a change in the physical properties of the catgut, and, I regret to say, in some respects this change is a slight disadvantage; but even then it is better than catgut which is used out of alcohol. At present it is of a deep brown, almost black color, and still retains the characteristic odor of iodine; it is perfectly smooth, not swollen, as one might expect from its immersion in an aqueous solution. The old Claudius catgut was exceedingly convenient to use, as it did not kink, nor curl up like catgut used from alcohol; but this newer catgut is somewhat stiff, like a fine wire, but it has no tendency to kink up, and particularly when it is used as a suture it soon becomes soft and straight. All in all, a long-continued use of it has proven to me its superiority also in this direction over alcohol catgut.

4. ABSORPTION.—Regarding this point, we have not made any recent observations. From a practical stand-point, I may say that the knots stay tight and do not tend to untie or loosen, and we have never seen any untoward symptoms which could be traced to a too early or too late absorption.

In this connection it might be of interest to mention that Dr. Bookman has attempted to gain experimentally some idea regarding its absorption, and he found that the von Bergmann catgut was completely digested in twenty-four hours in artificial gastric juice (pepsin and HCl); while on the other hand the same amount of iodine catgut was only partially disintegrated in seventy-two hours. From this we would be entitled to conclude that the iodine catgut is more resistant to absorption than the von Bergmann catgut, were it not for the fact that conditions in the body are different from the test-tube; in the latter we have a definite quantity of digesting fluid, but in the former we have leucocytes and constantly changing currents, which no doubt materially aid in absorp-

tion. In general, we may say that there is no material difference regarding the time of absorption between this and catgut prepared by other methods.

DISADVANTAGES.—Of these I know none, but, in order to prevent any misconception, it will perhaps be wise if I briefly mention those possibilities which might be raised against it.

The toxic effect of the iodine might cause some anxiety in the minds of some surgeons. There need be no fear on this account, because, first, even in the most extensive operation, *e.g.*, radical operation for carcinoma of the breast, the toxic dose can never be reached; second, because the iodine is divided so minutely that it quickly enters into a chemical combination with the salts of the body fluids, and forms only innocuous iodine compounds, etc., and third, because of late, after being so astonished at the almost marvellous antiseptic properties of the iodine solution, I have in a large number of cases used iodine solution for dressing wounds, using at each dressing large quantities of gauze dipped into iodine solution, but in no instance did I find even the slightest trace of irritation. (I may state here that I am now at work experimenting extensively with iodine gauze as a dressing, and thus far my experiments are so encouraging that I intend to publish the results at some future date.) For the reasons stated, I may also reply to the objections of those who fear that the iodine may act as an irritant upon the tissues.

The odor of the catgut and the staining of the linens are so trivial that they do not even merit discussion; particularly is this true of the latter, as by experience we have found that the stains are not permanent and readily disappear in the laundrying process.

I believe that our clinical experience and the experimental work as related in this paper fully justify the following conclusions:

1. The "dry" iodine catgut is absolutely sterile.
2. It is impossible to infect it by ordinary means.
3. Its imbibition with iodine is not sufficient to act as an irritant upon the tissues.

4. Its tensile strength is superior to raw catgut and to that prepared by the sublimate-alcohol method.
5. It is easily and cheaply prepared.
6. It is absorbed only after it has served the purposes for which it was intended.

REFERENCES.

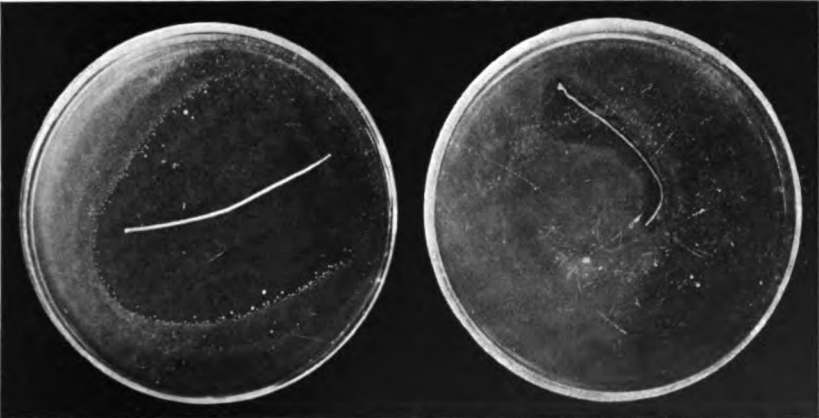
- ¹ Moschcowitz. New York Medical Record, May 14, 1904.
- ² Claudius. Deutsche Zeitschrift für Chirurgie, vol. lxiv, page 489.
- ³ Ibid. Deutsche Zeitschrift für Chirurgie, vol. lxix, page 462.
- ⁴ Martina. Deutsche Zeitschrift für Chirurgie, vol. lxx, page 140.
- ⁵ Von Hippel. Zentralblatt für Chirurgie, 1903, page 1301.
- ⁶ Johnson. Boston Medical and Surgical Journal, February 9, 1905.

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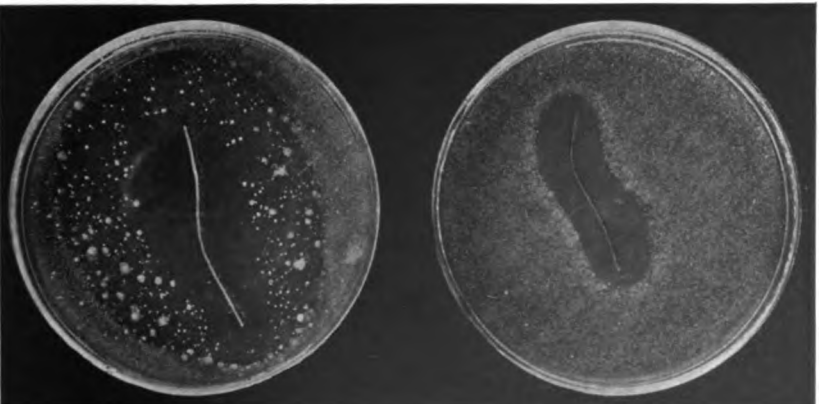
**No. 52. Agar infected with *Bacillus coli*,
with iodine gut on top.**

**No. 53. Agar infected with *Bacillus coli*,
with von Bergmann gut on top.**



**No. 54. Agar infected with *Staphylococcus aureus*,
with iodine gut on top.**

**No. 55. Agar infected with *Staphylococcus aureus*,
with von Bergmann gut on top.**



**No. 111. Agar infected with *Bacillus anthracis*,
with iodine gut on top.**

**No. 112. Agar infected with *Bacillus anthracis*,
with von Bergmann gut on top.**



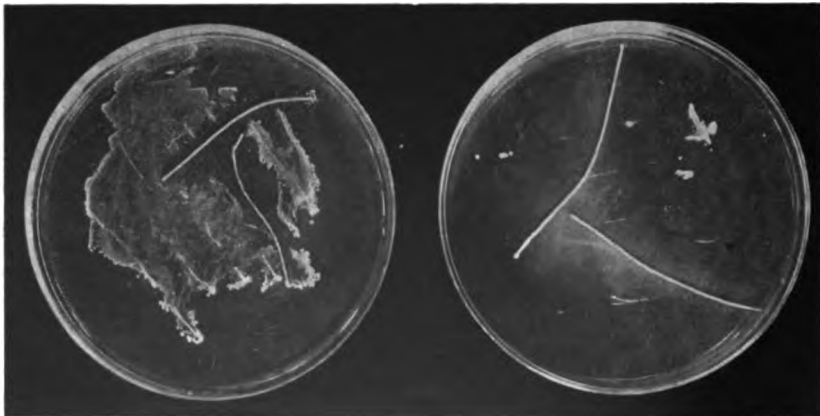
No. 56. Smears of *Bacillus coli* on agar, with iodine gut on top.

No. 57. Smears of *Bacillus coli* on agar, with von Bergmann gut on top.



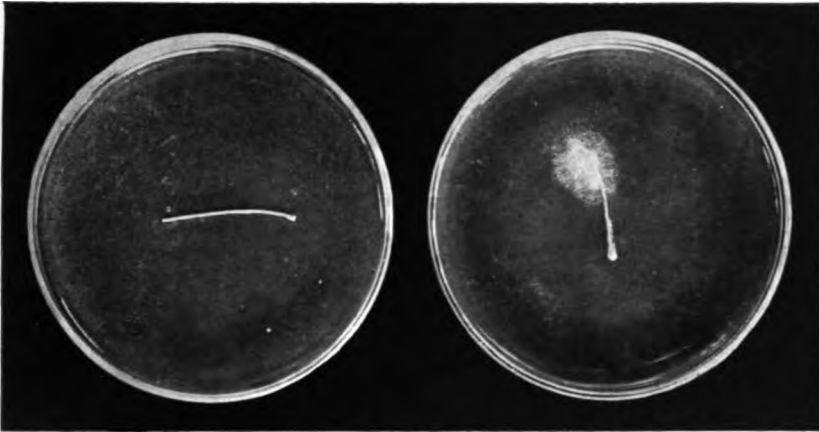
No. 58. Smears of *Staphylococcus aureus* on agar, with iodine gut on top.

No. 59. Smears of *Staphylococcus aureus* on agar, with von Bergmann gut on top.



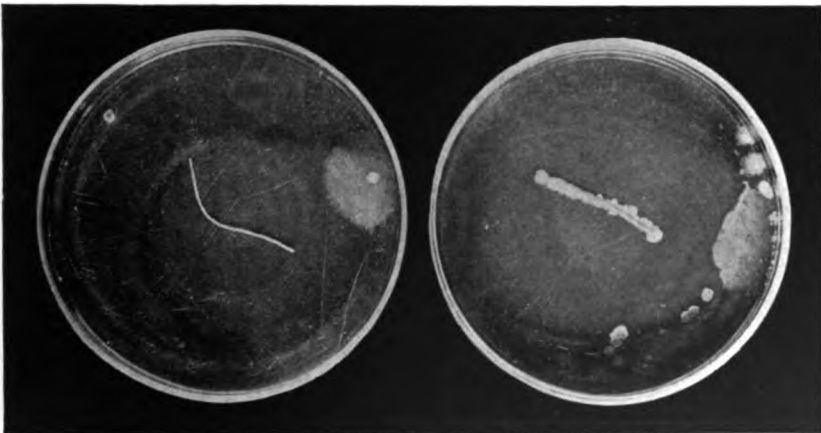
No. 69. Smears of *Staphylococcus aureus* on agar, with von Bergmann gut on top.

No. 68. Smears of *Staphylococcus aureus* on agar, with iodine gut on top.



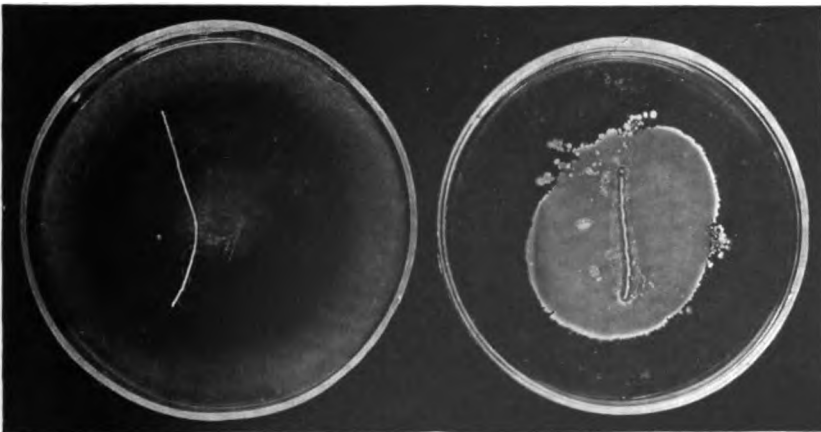
No. 74. Iodine gut infected with *Staphylococcus aureus* "dry" on agar.

No. 75. Von Bergmann gut infected with *Staphylococcus aureus* "dry" on agar.



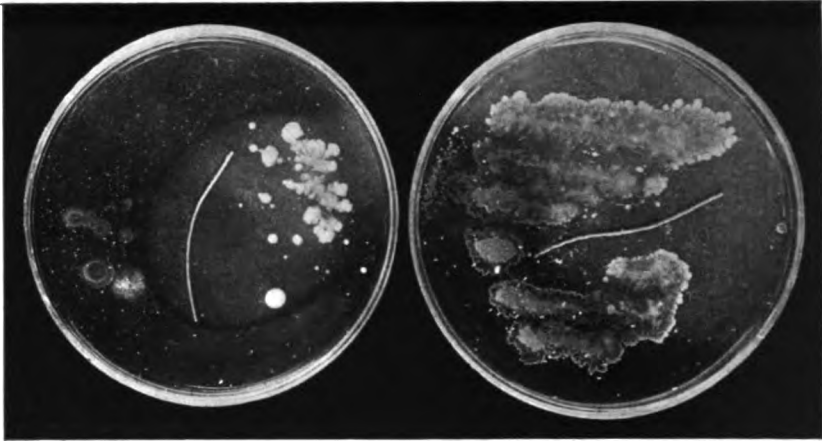
No. 127. Iodine gut infected with *Bacillus anthracis* "dry" on agar.

No. 128. Von Bergmann gut infected with *Bacillus anthracis* "dry" on agar.



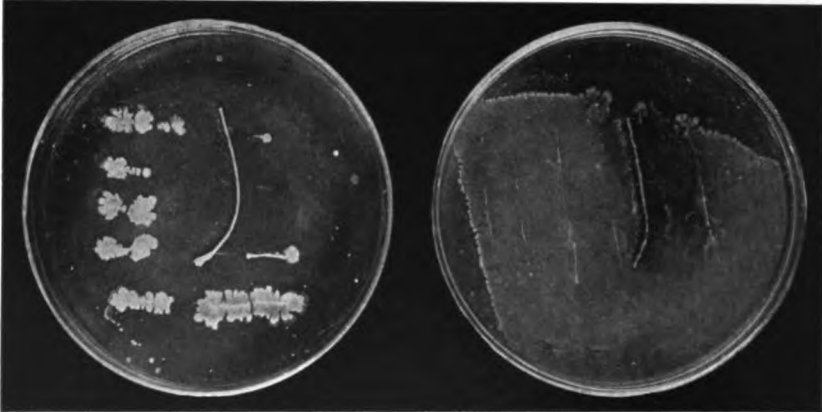
129. Iodine gut infected with *Bacillus subtilis* "dry" on agar.

No. 130. Von Bergmann gut infected with *Bacillus subtilis* "dry" on agar.



No. 93. Smears of *Bacillus anthracis* on agar, with iodine gut on top.

No. 94. Smears of *Bacillus subtilis* on agar, with von Bergmann gut on top.



No. 95. Smears of *Bacillus subtilis* on agar, with iodine gut on top.

No. 96. Smears of *Bacillus subtilis* on agar, with von Bergmann gut on top.



No. 72. Iodine gut infected with *Bacillus coli* "dry" on agar.

No. 73. Von Bergmann gut infected with *Bacillus coli* "dry" on agar.

"RED-LEG"—AN INFECTIOUS DISEASE OF FROGS.

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AND

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Having lost frogs in large numbers every autumn since 1897 from an apparently very virulent epidemic disease, we were led to investigate an unusually severe type of the trouble which destroyed two hundred of a stock of three hundred within two weeks in October, 1903.

In spite of some variations from the morphology as described by previous observers, the identity of the *Bacillus hydrophilus fuscus* as the essential etiological factor in the disease was definitely determined. In the course of our studies a number of observations were made both upon the pathological conditions found in the frogs, and upon the products of the bacterial cultures, which have not previously been noted. These facts, together with conclusions of practical interest, seemed sufficient reason to offer the following study in confirmation of the work of others, and in the hope that we may give assistance to laboratory workers who have suffered in a similar manner.

PREVIOUS STUDIES.—In an article by F. H. Russell,⁷ a very complete picture of the epidemic is to be found, including the results of autopsies and the cultural findings of the *Bacillus hydrophilus fuscus*. Russell concludes that nothing definite can be stated regarding the mode of infection, but believes the infectious material comes to the laboratory with the frogs and gains access through superficial skin lesions, and lastly, that Chicago water does not contain the infectious agent. In addition to these observations Russell determined the presence of two toxins as

the products of pure culture of the bacillus in bouillon; one having the physiological action of digitalis, the other resembling veratrin in effect.

An organism associated with this disease of frogs was first described as *Bacillus ranicida* by Ernst,⁷ and almost at the same time a bacillus resembling it in many respects was studied by Sanarelli,⁸ who found it to be pathogenic for warm-blooded animals as well as for frogs, and named it *Bacillus hydrophilus fuscus*. He states definitely that his *B. hydrophilus fuscus* is not identical with the *B. ranicida* of Ernst. Both observers were led to their studies because of the loss of frogs which they were using in their studies upon anthrax. The bacillus studied by us corresponds more closely to Sanarelli's than to Ernst's.

Ernst made a few inconclusive experiments upon the effect of different temperatures upon frogs inoculated with pure cultures of the bacillus, but is inclined to believe that the frogs are able to withstand the infection better at high temperatures (20° R.) than at low temperatures (7° R.). He also notes that *Rana esculenta* seems to be more susceptible than *Rana temporaria*. He lost most of his frogs in spring and summer. Sanarelli tested the portal of entry of the infectious agent by wounding frogs with a clean scalpel and putting some in a tank with sterile water and others in the local tap water. The latter became infected, the former did not. He found the bacillus in the water supply. He concludes that the bacillus is infectious and virulent at temperatures above 30° C.; that it develops quickly in warm-blooded animals; that the filtrate from pure cultures, when injected in quantities of the same volume as were used in causing fatal infections, gave no poisonous effect. He used indefinite doses of "several drops."

Trambusti¹⁰ also was led to his investigations because of losses among frogs in his laboratory. After identifying the bacillus and describing the clinical and pathological findings he studied (1) the physiological effect of pure cultures; (2) the physiological effect of the products of bacterial growth precipitated by alcohol; and (3) the effects of the substances soluble in alcohol.

The effect of (1) was to cause a tetanic condition of muscles and not paralysis. The effect of (2) was similar to the effect of caffen and veratrin upon muscles. The effect of (3) was paralysis.

Roger⁵ observed an epidemic in June and merely notes the presence of the bacillus in question and the typical pathological findings. He also confirms Sanarelli's observations upon mammals as well as upon batrachians.

Legrain,⁴ in 1888, described a gangrenous septicæmic infection among frogs, but with quite a different appearance and cause.

PRESENT STUDY.—At the time when our last epidemic was at its height, letters were addressed to a number of laboratories in this country and Canada, and, through the courtesy of the professors or their assistants in charge, twenty-one replies were received.* The name often given to the disease in these letters is "red-leg," and this is also the name used by the frog-catchers who state that the disease is well known to them and is seen very generally in the autumn months. The name is useful because it attracts attention at once to the most striking charac-

* We take this opportunity to express our gratitude for the courteous attention given by the following observers to our inquiries, whereby we obtained valuable information:

Professor C. W. Greene,	University of Missouri.
" W. P. Lombard,	" " Michigan.
" Isaac Ott,	
" Colin C. Stewart,	" " Pennsylvania.
" Arthur P. Brubaker,	
" James W. Warren,	Bryn Mawr College, Pa.
" Lafayette B. Mendel,	Yale University.
" W. R. Coe,	
" Wesley Mills,	McGill University.
" Gaylord P. Clark,	University of Syracuse.
" Winfield S. Hall,	Northwestern University Medical School.
" Theobald Smith,	Harvard Medical School.
Dr. W. B. Cannon,	" " "
Professor Graham Lusk,	Univ. and Bellevue Hosp. Medical College.
" George P. Dreyer,	
" Sidney P. Budgett,	Washington University, St. Louis, Mo.
" Theo. Hough,	Massachusetts Institute of Technology.
" George T. Kemp,	University of Illinois.
Dr. Elias P. Lyon,	" " Chicago.

teristic of the diseased frogs, the circulatory congestion of the belly and legs, varying from a faint flush to deep hæmorrhagic injection. The answers may best be arranged under the five questions asked in the circular letter:

I. Have you ever suffered from an epidemic disease among frogs?

Epidemics have been noticed, with characteristic lesions and clinical pictures sufficiently resembling those noted in our epidemic to warrant belief that they were of the same nature in and about Philadelphia and Bryn Mawr, Pa.; Cambridge, Mass.; Ann Arbor, Michigan; Chicago; Baltimore; Brooklyn; Syracuse; Montreal, Canada; Middletown, Conn.; Palo Alto, Cal.; and Columbia, Mo.

A few replies received from observers who had supplies from the same regions as those noted above, especially from Philadelphia and Chicago, state that they have never lost frogs from epidemic disease at any time of year, but this difference in the facts noted by adjacent observers may be accounted for possibly, first by the fact that these observers have had occasion to keep frogs in confinement but a short time, i. e., a week or two, during which time the manifestations of the epidemic are rarely seen. A second explanation seems to be found in the observation of certain precautions by these observers such as care in catching and cleaning and in protecting the frogs when caught from injury or infection or high temperatures.

II. What were the lesions noted, if any?

There was a general agreement as to certain features of the clinical picture and the character of the particular lesions most readily noticed.

Skin lesions, ecchymoses, and ulceration, not merely of the nose from abrasion on the side of the tanks, but of the belly and legs, were noticed by twelve observers. Three others noted ulcers on the feet and nose, with progressive sloughing of the tissues, but none of the characteristics of the epidemic under consideration. Edematous condition before death among almost all frogs dying with the ulcers and ecchymoses was noticed by five. By two observers, evidences of convulsive muscular action were seen slightly preceding, or at the time of, death.

III. At what time of year have epidemics been seen?

Ten observers state they have seen the epidemics in the warm weather of the autumn, three noting it also in the spring. One noted July and August to be the worst months, most of the others suffering losses chiefly in September and October.

IV. Were any etiological factors noted?

The following are some of the points noted which seemed to different observers to share in causing epidemics: injury during catching, exposure to high temperatures during shipment or in the laboratories, lack of food supply, crowded conditions in the tanks, dirty tanks, tanks with linings of iron, zinc, rough rocks, or with gravel bottoms.

The clinical picture observed in our epidemic was very definite

and uniform throughout the months of October, November, and December; the variations which at first seemed essential differences were later found to be indications of the varying severity of the disease in different individuals or in groups of frogs.

The first thing about the diseased frogs which attracts attention is their sluggishness, which is often so great that they will not move to avoid a hand in the tank. They huddle together instead of jumping away. Chemical and mechanical irritation elicits but slight response. This persists and increases until there is complete failure to respond to stimuli, although if the thorax be opened the heart will be found still beating. On making kymographic tracing with the sciatic-gastrocnemius preparation the curves resemble fatigue curves, in their diminished height, prolonged relaxation period, and increased latent period. This phenomenon was so marked that it was difficult to demonstrate to a student class fatigue by comparison with fresh muscle, so immediately did the muscle fail to respond to stimuli. Distension of the lymph sacs with serum gives them a sodden and bloated appearance, making the sluggishness seem due to weight as well as to paralysis. Dulness of the coat is striking in *Rana tigrina* and *R. viridis*, the markings being less distinct, while the colors are paler than in healthy frogs under the same conditions. Congestion of all the ventral surfaces of the body from jaw to tip of hind legs is usual to greater or less degree. The head and trunk are at a much more acute angle with the basis, this position being exaggerated until just before death, when the lower jaw touches the ground and all the legs are sprawling. The attitude is such as to cause the frog to breathe under water for some time before death if there be water only to the depth of half an inch in the tank.

In several instances muscular spasms or tetanic convulsions were noted before death.

The clinical picture given by Russell⁷ is similar, but he speaks of muscular spasms before death as appearing frequently, while we noticed them in only about one per cent of all deaths. The results of our autopsies were also similar to those of Russell.⁷ Seventy-five frogs of one hundred and fifty which died within

three to five days of their arrival from the marshes of the Delaware and Schuylkill rivers on October 10, 1903, were examined. The lesions were remarkably uniform, but as the epidemic diminished upon the advent of colder weather, we found all grades of severity of lesions, and of suddenness of onset and duration of illness. The skin lesions consisted of hæmorrhagic papules and occasional vesicles, thirty-three per cent of the frogs showing especially large hæmorrhagic exudates under the skin at the joint of the upper and lower extremities, these latter appearing always in the frogs in which other lesions were most severe. This latter class of cases showed no recoveries, death always following rapidly after the appearance of the periarticular hæmorrhages. The extent of the hæmorrhagic skin lesions might well be taken as a measure of the severity of the disease. A few showing diffuse or petechial hæmorrhages into the skin from the mandible to the toes, varying from a slight flush to deep injection, and general hæmorrhages with ulcers, recovered; while a larger number, with a small area of congestion and a few vesicles seemed none the worse for their sickness a couple of weeks later.

The dorsal and ventral lymph spaces were distended with colorless or blood-tinged fluid, according to the severity of the attack, giving the bloated appearance so often noted, and to be explained by the grave changes in the circulating blood. In some of the frogs autopsied within an hour or two after death the heart-blood was found almost colorless and to contain very few red cells.

The tongue was found occasionally flecked with hæmorrhages. Muscular, intermuscular, and periosteal hæmorrhages were often extensive in the hind legs, accompanied by a softening of muscular tissue.

The lungs were the seat of no apparent change, but were in most instances invaded by either the *Distomum cylindraceum*, or by a small ascarid in large numbers, the former sometimes being encysted in the wall of the lung.*

* This is probably the *Rhabdomena* (*Ascaris*) *nigrovenosum* described by Miss Sheldon⁹ in *The Cambridge Natural History*. We have found no other description of a parasite similar to the one seen by us found in the lungs of

When the ascarids were put into a very weak saline solution, they at once became violently contorted and gave out into the fluid countless eggs, through the thin shells of which the embryos could be seen twisting and turning. A still better view of the embryos was had by putting them in glycerine, when the shell previously cloudy though translucent, cleared.

The heart was sometimes pale, but oftener appeared to be the healthiest organ in the animal.

The stomach was uniformly distended with viscid, ropy mucus, and was markedly congested. The intestines were normal except for congestion which was frequently noted, and occasional prolapse of the anal end of the gut. The spleen and liver were soft and mottled.

Specimens taken from the lymph spaces and abdominal cavity of three or four gave uniformly pure cultures.

Essentially the same lesions were found, though in varying degrees of severity in frogs (*R. viridis*) previously healthy and coming from New Rochelle, N. Y., and (*R. tigrina*) Chicago, Ill., infected by living in the same tanks with the diseased frogs. The larger frogs succumbed much less readily than the small ones, and the large and vigorous *R. tigrina* from Chicago proved much more resistant than other varieties under the same conditions, and showed no deaths within a month after their receipt.

In November we secured a lot of healthy *R. esculenta* and *R. tigrina* from Ithaca, N. Y., and of these none developed the disease except on inoculation.

During October, November, and December, 1903, our frogs were received as follows:

October—300 from Philadelphia, of which 200 died of the disease. 70 were used before death, and 30 lived without showing any lesions. These consisted chiefly of *R. esculenta*, among which were a few of the species *R. viridis*.

November—25 from New Rochelle; a few of these died, but only one with lesions resembling those described above. All were *R. viridis*.

November—50 from Chicago, of which 10 died, although put in cleaned

frogs, but we have not seen the free sexually mature form of the alternating generation, as mentioned in the above description.

tanks, the rest being used for our other studies. All were *R. tigrina*.

December—50 from Chicago, of which 6 died of the disease, the others being used for inoculation and other studies. All were *R. tigrina*.

December—45 from Ithaca, N. Y., of which none died except those inoculated with the organisms of the disease. They consisted of *R. esculenta* and small *R. tigrina* in about equal numbers.

As the frogs were received they were put in soapstone sinks, each lot separate from the others, and each with an independent supply pipe and exit for water. The city water was used altogether and allowed to trickle from the sink faucet through the galvanized iron wire gauze covering the top of the sink. The sinks were occasionally scrubbed with soap and flushed out with boiling water. The water usually ran cool, but often the overheating of steam pipes raised the temperature to 35° C. None of the frogs was fed at any time.

INOCULATION EXPERIMENTS.—Three sets of inoculation experiments were made, first (A) to test the specific character of the bacillus; second (B), to see if temperature had any effect upon the course of the disease; and third (C), having found very striking effects from cold, to test the extent of the effect of keeping inoculated frogs in cold storage. The results follow.

Series A.—Four frogs (*R. tigrina*) from Chicago, apparently healthy, were inoculated with one-thirtieth of a twenty-four-hour agar culture of the *Bacillus hydrophilus fuscus*, obtained in pure culture from an early case of the disease and kept at room temperature. One was killed at the end of twenty-four hours because it presented the typical appearance of oedema, dullness, red-leg, and superficial ulceration. Autopsy showed the usual pathological appearances in the viscera and lymph. Smears of the blood showed advanced degenerative changes in the red cells, and in a few of the cells single and occasionally double bacilli were found; a few bacilli were also found extracellular. Blood cultures showed pure growth of the specific bacterium. Two others which died in forty-eight hours presented the same picture as the one described above, but the blood changes were more marked. The fourth died in fifteen days without oedema, but

with degenerative changes in the viscera and marked blood changes. This animal had thirty-eight ascarids in the lungs. It also showed the specific bacterium in the blood from the spleen but less abundantly than the others.

Series B.—Five frogs (*R. esculenta*) from Ithaca were inoculated on December 27th with the same strength of emulsion as was used in series A. Two were kept at room temperature and three were put in a cold-storage closet, where the temperature was never over 5° C., and always went down to 0° C. at night. The two at room temperature died within eighteen hours, and on autopsy showed great oedema and visceral degeneration, and the following blood changes: The lymph of the ventral sac was abundant and pink; no red blood flowed anywhere on incision; on opening the heart no red blood appeared; on examining a fresh smear from the heart-blood, diligent search failed to show more than one or two red cells in a field; smears stained with Jenner's stain or fixed in methyl alcohol, and stained with methyl azure and eosin, showed abundant leucocytes and many more stained nuclei of red cells, these being apparently all that were left to represent the red-blood cells.

The other three frogs were kept in the cold until January 16th, and at no time did they show the slightest sign or symptom of disease. They were alive and active on January 30th, having been kept at room temperature since January 16th.

Series C.—Twenty-four frogs were inoculated on December 29th with five minims each of a twenty-hour agar-agar slant culture at 37° C., suspended in 5 cc. of normal saline solution. The injections were all made into the dorsal lymph sac. In other words, approximately one-thirtieth of an agar culture was used. The twenty-four frogs were selected as follows: twelve large frogs of species *R. tigrina* (class A) from Chicago, among which there had been no disease for two weeks previously. Six small frogs of species *R. tigrina* (class B) and six of species *R. esculenta* (class C), both sets from Ithaca, N. Y., among which there had been no disease since their receipt at the laboratory. Four of the twenty-four (two from class A and one from each of the other two classes) were kept at room temperature as controls.

The other twenty were kept in the cold-storage closet under the conditions above described.

At the end of twelve hours the four controls were still alive, though dull and swollen. At the end of twenty-two hours three were dead, one of class A and the two of classes B and C. The other of class A died in twenty-seven hours. Except in the case of the last frog, which showed milder lesions, the findings were those described in the severe cases above cited.

The skin lesions were of the hæmorrhagic and serous character, but there were muscular hæmorrhages also. The blood was almost colorless, and only a few scattered red-blood cells were found. Bacilli were numerous in the lymph and blood. There were two or three of the ascarids and distoma in the lungs of each of these frogs. Of the two removed from the ice-box after a sojourn of four days, both were in perfect health, but they unfortunately escaped from the jars and died, one two days, the other six days, later, apparently as a result of drying upon the floor, as lesions of the disease were absent and no bacilli were found in smears from the lymph and blood.

Of the twenty frogs kept at low temperatures (0° – 5° C.), some were allowed to remain continuously in the ice-box, and of these none died at any time.

The others were removed from the ice-box after a sojourn varying from twelve hours to thirteen days, and were then kept at room temperature. The results of this experiment follow:

Those removed from the ice-box after a sojourn of twelve hours died twenty-four hours to three and one-half days later.

Those removed from the ice-box after a sojourn of twenty-four hours died twenty-four hours to three days later.

Those removed from the ice-box after a sojourn of thirty-six hours died thirty-six hours to three and one-half days later.

Those removed from the ice-box after a sojourn of forty-eight hours died two and one-half to four and one-half days later.

Of these the one that died four and one-half days later escaped from the jar and was found dried and shrunken upon the floor, entirely lacking any of the general appearances or lesions seen in the others which died of the disease.

Of those removed from the ice-box after a sojourn of three days, one died seven days later, the other showed no appearance of disease and lived for several weeks.

Of those removed from the ice-box after a sojourn of five days, one died nine days later and one never showed any signs of the disease.

Of those removed from the ice-box after a sojourn of seven days one died nine days later, the other never showed any signs of disease.

Those removed from the ice-box after nine, eleven, and thirteen days showed no signs of disease at any time thereafter.

In other words, of the entire twenty subjected to low temperatures, after inoculation, the nine removed from the ice-box after a sojourn of four days or less showed seven deaths with lesions of the disease, one death without lesions, and one recovery.

The eleven removed from the ice-box after a sojourn of four days or longer showed two deaths, with lesions and bacilli in the blood or lymph, two deaths from accidental exposure and drying, seven recoveries. The last seven showed no signs of disease, and were alive and well thirty-two days after inoculation, although kept at room temperature continuously since removal from the cold. Of those which died, all removed from the cold under three days showed lesions of greater or less severity, and the bacilli were found present in all but one. One frog recovered after a sojourn of only three days in the cold, and the three placed in the cold chamber for only twelve hours lived longer by twelve hours to two weeks than any of those left entirely at room temperature.

The controls (i. e., the four inoculated frogs kept at room temperature) all died, while of the twenty inoculated frogs subjected to low temperatures 50 per cent recovered or died accidentally without the lesions of the disease. Of the deaths among these twenty, 60 per cent occurred in frogs which had been kept in the cold thirty-six hours or less, the proportions of recoveries increasing with the length of time they were kept in the cold.

The water in the jars in which the frogs were kept in the ice-box was always partly frozen during the night, the frogs' heads usually being embedded in the surface film of ice in the morning. The ice melted during the day as the temperature of the chamber rose (i. e., from 0° – 5° C.), because of the frequent opening of the doors in daytime.

An observation, which was frequently made in the animal house in which the frogs were usually kept, was that if by acci-

dent or intention the steam heat was allowed to warm the building to 20° C., or if the water running over the frogs in their sink ran warm (30° C.) for a day because of over-heating of adjacent steam-pipes, the spread of the disease was very rapid and fatal among the frogs which had previously shown but occasional cases at intervals of several days.

If the room was warm enough to be healthy for rabbits and guinea-pigs, it was too warm for infected frogs. The nearer the temperatures of the room and water were kept to freezing the healthier were the frogs.

An opportunity was afforded in September and October, 1904, to test on a large scale the efficiency of cold in the treatment of "red-leg." Six hundred frogs of species *R. tigrina* were received from Chicago on September 1st, and were kept in soapstone sinks at the laboratory temperature. For ten days they appeared healthy, and only occasional deaths from injuries or accidents occurred. Suddenly they began to die in large numbers, as many as 55 being found dead in one day. The gross appearances were identical with those described in previous epidemics, and a bacillus in pure culture isolated from the lymph and the blood proved to be *Bacillus hydrophilus fuscus*. The average daily loss by death for the week before we made our test was twenty-six frogs.

On September 25th 304 frogs remained, of which number 144 were put in a zinc-lined tray, 2 x 3 feet, in a cold chamber, with temperature varying from 1° C. to 6° C.

On September 26th one death occurred, due to freezing, as the frog was embedded in the ice, but this animal showed absolutely no lesions and inoculations from the blood and the lymph gave sterile plates. Of this lot no others died, the tray being left meanwhile in the ice-box until October 13th; while among the 160 frogs left in soapstone sinks at laboratory temperature, the deaths continued at the rate of ten a day for three days, when 124 of the remaining healthy ones were put in the ice-box. The next day one of these was found dead and showed the typical lesions, but after that there were no further deaths.

During the week following on one occasion the temperature of the cold chamber rose to from 6° C. to 9° C. and remained at

that point for twenty-four hours, and the following morning three frogs in each of the two lots were found with marked lesions. The six were removed to separate vessels, where they lived from one to two weeks. In other words, for the week of September 19th to 25th, when about 500 frogs were kept at room temperature, our loss was 182, and for the week of September 28th to October 5th, when all our frogs (268) were in the ice-box, our loss was one from red-leg, and six already diseased frogs which lived one to two weeks.

Observations on the Blood.—Two further observations were made to control our work: one concerning the presence of the protozoan *Drepanidium* in the red-blood cells, the other with reference to the anæmia and leucocytosis present in the frogs dying from the disease.

Several of the frogs received in October, 1903, from Philadelphia which were examined showed in stained smears of the blood, in addition to the abundant typical bacillus, a marked leucocytosis and the presence of a hæmatozoan parasite in the red cells. Comparison with the slides and drawings of Dr. Langmann³ given in his paper on the subject showed the organism to be the *Drepanidium* he described.

Subsequent search for this organism, to test its association with some of the lesions or characteristics of the disease, showed that it was present in only two per cent of the Philadelphia frogs and in one per cent of the frogs from other sources. There was no reason to consider it in any way related etiologically to the disease in question. This is in accord with the observations of Dr. Langmann, who found this hæmatozoan parasite to be without effect upon the life history of snakes.

In regard to anæmia among the frogs, two distinct causes were found for the severe grade present in a number, one of which may properly be classed as a predisposing factor in some cases, and the other as a direct and constant result of the bacterial infection.

The normal red-cell count in *Rana temporaria*, according to Rollett⁶ and Bethe,⁷ is 393,200 per cubic millimetre. In a number of apparently healthy frogs of different kinds the red-

cell count was found by us to vary from 354,000 to 500,000 per cubic millimetre. In a number of frogs with poor musculature and dull skin, but without any lesions of "red-leg" or any leucocytosis, the count varied from 114,000 to 354,000 per cubic millimetre.

In these frogs we found many distomes in the lungs. These parasites live upon the red-blood cells of the frog, which they suck from the pulmonary capillaries. On spreading a distome out upon a slide and compressing it slightly with a cover-glass, the intestine can be seen loaded with the oval nucleated red cells of the frog. Estimating the length of the canal by use of a micrometre scale, following the contortions of the tube, and then counting the number of red cells in a given straight length of the tube, the number of red cells in a single parasite was found to be 300,000. As these parasites were found in some frogs to the number of twenty and over, a total of at least 6,000,000 red cells may be estimated as having been drawn from a frog at a given time.

The total blood bulk of the frogs varied from 0.75 cc. to 1.5 cc. Taking 500,000 red cells per cubic millimetre as a basis of calculation, we have an estimated total number of red cells of 375,000,000 to 750,000,000 per frog, of which 1.6 per cent to 0.8 per cent would be at one time in the digestive canal of the parasites, whose very active digestion makes the process of erythrocytic destruction rapid and progressive.

These parasites were found in healthy frogs, and they were absent in many of the diseased frogs. Thus their presence is not a constant factor, but it seems probable that the anæmia they cause might diminish the resistance to infection or prevent recovery when infection has occurred.

The presence of the ascarid even in numbers up to twenty-six in a single animal appeared to have no relation to any morbid state of the frog. In many instances we found both kinds of parasites in one or both lungs of the same frog, whatever the source of the supply, but the occurrence of the distome was most common in the first supply of Philadelphia frogs which we received.

The constant occurrence of marked and severe blood changes

in the frogs dying of "red-leg" has already been noted, and it needs merely to be repeated that in the severer infections a careful search is required to discover a single red corpuscle in fresh and stained smear preparations of the blood. This observation led us to make a few experiments upon the effects of the filtrate from broth culture grown for a week at 37° C. The filtrate had no effect upon the red corpuscles; but a very different picture was presented by a hanging-drop preparation of a 1-10 saline dilution of normal frog's blood, and an emulsion of a twenty-four-hour agar-agar culture of the bacillus. From being distributed evenly through the drop, the very motile bacilli were, in the course of two hours, seen in clustered masses about the red cells. In three to four hours the cells showed irregular outlines and appeared paler. In ten to twelve hours nothing appeared to represent the cells but the nuclei about which were the now sluggish groups of previously actively motile bacilli. The three facts so often noticed at autopsy, namely, the severe laking of the blood, the presence of numerous isolated red-cell nuclei, and the almost total absence of red corpuscles in the severer cases, find a plausible explanation in the destruction of the red cells, as observed in the hanging-drop preparation.

It must be noted, however, that the great excess of bacilli in proportion to the red-blood cells in the hanging-drop preparation is in marked contrast to the actual conditions observed in the blood of infected frogs. And further, it should be explained that even though the filtrate has no effect *in vitro* upon the red corpuscles of the frog, it must be granted that there is a possibility that the products of the growth of the bacilli in the body may differ from those produced under artificial conditions and tested upon red-blood cells in a test tube.

MORPHOLOGY AND BIOLOGY OF BACILLUS HYDROPHILUS FUSCUS.—The bacillus isolated from the frogs affected with the so-called "red-leg" has striking biological characters. The following is a description of the morphology, and of the pathogenic and biochemical properties, of the bacillus.

Morphology.—As is the case with many bacilli, the morphology varies considerably, according to the source from which it has

been obtained. The tinctorial properties of the organisms likewise vary, depending upon the stains employed for their demonstration.

Thus in the blood of the frog the bacillus is a fairly thick rod, exhibiting, by Jenner's blood stain, marked bi-polar and other irregularities of staining. A form commonly seen in the fresh blood smears is a shovel-shaped bacillus. The square ends of these forms appear strikingly fringed, and it is by these ends that they often appear in apposition as diplobacilli. With the ordinary dyes, however, the bacterial plasma is uniformly colored. The rods appear single or as diplobacilli, and they are seen within both the white and red corpuscles, in the former being present in considerable numbers, especially in frogs about to die. Just before death, and in the blood of dead frogs, the bacilli may be present in large numbers.

In young cultures on all media shorter and longer rods predominate, while coccoid forms and diplobacilli are also present. The bacillus may be said to be somewhat thicker than the typhoid bacillus, the rods being straight, and on the average the length is five to six times the breadth. In older agar cultures—after four days to several weeks—and on potato the rods remain short and thick, although filaments and forms staining poorly are met with occasionally in abundance. After several days to a week's growth on coagulated ox serum, the organism becomes markedly filamentous and long rods and bizarre involution forms are frequent.

Young cultures stain intensely with all the ordinary dyes. No spores are developed. The bacillus is very actively motile, and does not retain Gram's stain. The thermal death point was not ascertained.

Pathogenicity.—The marked pathogenic action of the cultures on frogs has been described above. On guinea-pigs emulsion of young agar cultures exerts a toxic and rapidly fatal action, one-tenth of a twenty-four-hour culture introduced into the peritoneum or subcutaneously producing death within twelve hours. The site of inoculation in the subcutaneous tissue is the seat of hæmorrhagic infiltration. Guinea-pigs inoculated in the peritoneum

develop a marked peritonitis with blood-tinged exudate. Cultures taken from the heart's blood of guinea-pigs just after death develop a few colonies. The fatal action is thus toxic and septicæmic.

Rabbits, on the contrary, are not susceptible. One-tenth of a 24-hour agar culture produces a slight and transient indisposition, when inoculated by way of the ear vein; and if by way of the skin small abscesses which tend to heal.

The pathogenic action on other animals was not determined.

Cultural Characteristics.—The growth is rapid and abundant upon the usual media, the optimum temperature being about 37° C., although growth is abundant at room temperature and at temperatures as low as 12° C.

The colonies upon agar and gelatin present no diagnostic characters.

The plates on 12 % gelatin, "2 % acid to phenolphthalein," develop colonies which are yellowish by transmitted light, and irregularly marked. When small and young, they are pale and slightly stippled. Liquefaction is complete in two days in the cold room, and well-isolated large colonies cause advanced liquefaction within two days.

The superficial colonies on agar are whitish, somewhat raised and moist, and are coarsely stippled. The circumferential portions of the colony are pale and have even borders, the central portion is decidedly yellowish and is marked by round aggregations. The deep colonies are round or oblong, deep yellow in color, and irregularly lobulated.

Young cultures are odorless. Old cultures, however, acquire a rank odor.

Gelatin Stab.—A small globular-shaped area of liquefaction develops beneath the surface of a gelatin stab culture. The liquefaction increases slowly in extent and is not complete for several weeks. A whitish pellicle is soon formed and a heavy, whitish deposit settles out promptly. No greenish tint was observed. Gelatin stabs grown for two days and then melted remain fluid at room temperature. After three days' growth the gelatin remains fluid when placed in cold storage

Milk.—Milk is completely clotted in twenty-four hours at 37° C. Liquefaction of the clot is apparent within four days, and is practically complete in ten days. At room temperature clotting and liquefaction occur more slowly than at incubator temperature.

The rapidity and character of these processes vary somewhat in different cultures.

In litmus milk, "1.5 % acid to phenolphthalein," a marked acid reaction promptly develops. The coagulum, instead of being solid, may be finely divided, and then settles out promptly, leaving the fluid clear. The clot never completely disappears, even after four weeks, and may or may not retain its acid color.

In milk rendered alkaline (0.2 cc. $\frac{N}{4}$ NaOH to 10 cc. milk) the reaction becomes acid, and a finely divided clot appears in two days at 37° C., which is soon liquefied, the fluid becoming clear. In milk rendered acid (0.2 cc. $\frac{N}{4}$ HCl and 10 cc. milk), the same succession of changes is observed, as also in similar sets of milk-tubes grown at room temperature, the reaction in the latter case taking place more slowly.

The Enzymic Action of Filtrates of Broth Cultures upon Milk and Gelatin.—The sterile filtrates of broth cultures in milk bring about changes similar to those caused by the living bacillus.

The following tests were made:

Litmus milk 10 cc. + 1 cc. broth filtrate (1.3 % acid; culture grown twenty-eight days at 37° C.). Soft clot third day at 37° C., fifth day clot settles out and liquefaction proceeds slowly. After four weeks a small clot sticks to the sides of the tube, the fluid being left perfectly clear.

In litmus milk, to which 0.2 cc. $\frac{N}{4}$ NaOH was added, decolorization begins promptly, coagulation occurs at two days, clarification is complete on the fifth day. The control milk-tube, to which no filtrate had been added, showed the same changes, due to alkaline clarification of the milk, after the fifth day.

In milk rendered strongly acid by addition of 0.2 cc. HCl, clotting and liquefaction of the clot occur as in the milk without addition of acid.

In milk-tubes kept at room temperature clotting is delayed until the tenth day, and the liquefaction is much less extensive and complete than in the tubes kept at 37° C.

The peptonizing action of filtrates on gelatin is rapid and complete.

1 cc. filtrate (growth of four weeks) added to 6 cc. of melted gelatin (12 %) produces permanent liquefaction in two days, 0.5 cc., in five days, while 0.1 cc. requires several weeks to produce slight softening of the gelatin.

The liquefying action of the filtrate (1 cc.), when added on top of a column of gelatin, is much slower, one inch of gelatin being rendered fluid in a week, the action being complete after four to five weeks.

The peptonizing action of the filtrate is greatly accelerated in the incubator.

The filtrates of young broth cultures are apparently more active than those obtained from older cultures.

The filtrate of a three-day broth culture grown at 37° C., reaction 1.75 acid to phenolphthalein gave the following action upon milk:

5 cc. filtrate added to 10 cc. milk, twenty-four hours at 37° C., produced a marked clarification of the milk, in three days the fluid becoming perfectly clear, a small coagulum being left at the bottom of the tube.

1 cc. of the filtrate added to 10 cc. milk causes a solid clot in twenty-four hours, which becomes peptonized in a couple of days. The enzymic action of the filtrate at room temperature is considerably slower than at incubator temperature.

Gelatin, 5-7 cc., to which when fluid 1 cc. of the filtrate is added, remains permanently fluid at room temperature.

Broth.—Marked turbidity occurs within twenty-four hours, and flocculi and a heavy pellicle soon develop.

Potato.—On potato (natural reaction 0.5 % acid to phenolphthalein) at room and incubator temperature the growth is abundant, wet, slightly raised, and yellowish. This yellow color changes in the course of a few days to a reddish hue, assuming later a rusty-colored sputum tint.

In four weeks the potato is covered by a heavy and dull brownish-colored growth, the upper and dry portion of the growth presenting a greenish, metallic lustre.

On the same potatoes rendered alkaline by 0.2 % NaOH, the growth never turns reddish in color, but is abundant, the early citron color turning slowly into a brownish gray.

On acidified potatoes (HCl) the growth is scanty and invisible.

The pigment production on potatoes was found to vary considerably, the reddish tint not appearing constantly, but being much more marked in those potatoes, especially when dry, inoculated from cultures recently isolated. On dry potatoes the

growth was markedly raised and wrinkled, and of an intense reddish brown or terra-cotta color.

The red pigmentation was observed only on the potato.

Slant Agar.—The whitish, moist streak spreads rapidly, and the growth assumes a yellowish tint in the course of time. The yellow pigment production is more marked in the early than in the later subcultures. The yellowish pigmentation of the early cultures became intense when grown for several weeks.

Coagulated Ox Serum.—Abundant whitish growth; no pigment formation; gradual softening of the serum, the liquefaction in the tubes kept at 37° C. being concealed by the evaporation.

Fermentative Action upon Sugars.—When freshly isolated from frogs and tested upon various sugars (Theobald Smith's sugar-free broth with addition of 1 % of sugar), the bacillus fermented mannite, dextrose, and saccharose with the production of acid and gas. The gas production, however, was slight, a large bubble of gas developing in the course of several days at 37° C. Both arms of the fermentation tube became markedly turbid.

Lactose was never fermented with gas production, although the growth in both arms of the fermentation tube was abundant.

After cultivation for a few generations upon agar, the bacillus lost its property of fermenting sugars with gas production. The property of growing in the closed arm of sugar-free broth in Smith's fermentation tube, as shown by the turbidity after twenty-four hours' growth at 37° C., was retained, but in a lesser degree.

The growth in the open arm of the tube containing sugar-free broth and lactose broth is abundant, a heavy, whitish deposit settling out in a few days.

The growth in the open arm of the tubes containing dextrose, mannite, and saccharose broth is not nearly as abundant as in the lactose and sugar-free broth, the cloudiness in both arms being practically similar.

An examination of the following table indicates that the most probable explanation of the slight growth in the open arm is found in the production of acid in the fermentation of the sugars

(dextrose, saccharose, and mannite), which acid inhibits further growth.

TABLE TO SHOW THE AMOUNT OF ACID PRODUCED IN SUGAR-FREE BROTH (1 % ACID TO PHENOLPHTHALEIN), TO WHICH 1 % OF VARIOUS SUGARS HAS BEEN ADDED.

<i>Grown 24 hrs. at 37° C.</i>	<i>7 days (idem.)</i>	<i>4 weeks (idem.)</i>
Open arm, 1 %	1.1 %*	Not tested.
Closed arm, 1.3 %	2 %	" "
<i>1 % Lactose</i>		
Open arm, 1.1 %	1 %*	Not tested
Closed arm, 1.3 %	2.2 %	2.3 %?
<i>1 % Dextrose</i>		
Open arm, 2.2 %	2.7 %	4.1 %
Closed arm, 2 %	1.8 %	3.2 %
<i>1 % Saccharose</i>		
Open arm, 2.3 %	2.8 %	4.4 %
Closed arm, 1.9 %	2.2 %	3.1 %
<i>1 % Mannite</i>		
Open arm, 2.1 %	2.5 %	Not tested
Closed arm, 1.1 %	2.2 %	" "

Fermentation Reactions in Hiss's Serum Media.—† Hiss's, 1 % dextrose-, mannite-, saccharose-, maltose-, dextrin-and-starch-, litmus serum water (1-3) becomes clotted firmly in twenty-four hours at 37° C.

The clot in the dextrose tubes remains acid and solid for weeks. Softening of the clot begins in the other sugar serum waters after three days, but never becomes liquefied to any extent, except in the dextrine tube. The clot is an acid clot.

In 1 % lactose and 1 % inulin-litmus serum water (1-3), the color, as in the plain sugar-free litmus serum water, remains unchanged, no acid reaction being developed. The serum water becomes opaque, and finally solid in four days. Softening slowly takes place, the clot being almost liquefied in three weeks, and the litmus is decolorized. The clot in these tubes is a "sweet" clot due to a rennet-like ferment.

* Rose tint on adding phenolphthalein.

† Hiss, *Centralb. f. Bakt.*, 1902, xxxi, 302; *Science*, 1902, March 7, p. 367; *Medical News*, New York, 1903, February 14, p. 6.

The filtrates of broth cultures have no action upon the various sugar serum water media, thus 1 cc. of a 28-day filtrate leaves the serum (5 cc.) unchanged after several weeks at 37° C.

From these observations the conclusion seems justified that the fermentation of the sugars is produced through the action of an intracellular bacterial ferment, that is, one which is not extracted or set free, or at least not in sufficient quantities, during the growth of the bacillus in the broth cultures, and is thus absent in the filtrates. In other words, the fermentation of the sugars with acid production is induced only through immediate contact of the living bacillus with the sugar, the filtrates leaving the reaction of the litmus unchanged, as seen in serum water tubes.

In marked contrast to these ferments are those soluble ones we have already described, which peptonize gelatin, and which coagulate and subsequently liquefy or peptonize milk and serum.

We do not know definitely whether the rennet-like ferment and the peptonizing ferment are distinct. The bacillus does not split urea or asparagin with acid production, as 1 % urea and asparagin litmus peptone solutions are unaffected.

Reduction of Nitrates. Indol.—The bacillus rapidly reduces nitrates to nitrites, and indol is slowly produced in Dunham's peptone solution.

An intense color reaction is developed in the nitrate peptone solution in which the bacillus has been grown for twenty-four hours at 37° C., when tested with the sulphanilic or "naphthylamine test." After five days' growth the nitrites are completely reduced, no color reaction being now obtainable.

Growth for twenty-four hours in Dunham's peptone solution develops a marked reddish color on addition of concentrated sulphuric acid, while in tubes grown for two days no color reaction develops. After four days' growth at 37° C. a faint rose tint develops on addition of sulphuric acid and the nitrite solution. The color reaction becomes intense, when the tubes have been grown for from two to four weeks. The deep cherry-red color then obtained varies somewhat from the usual tint obtained with the indol test.

THE IMMUNE BODIES DEVELOPED BY ADAPTATION OF RABBITS TO THE BACILLUS.—Agglutinins and precipitins are developed in rabbits adapted to emulsions of living bacilli, as well as those inoculated with filtrates of broth cultures. Thus in a rabbit inoculated with living organisms an agglutination limit of 1-12,500 in two hours ("microscopical clumps") was obtained, the precipitation limit being slightly above 1-10 (28-day filtrate).

We were unable to demonstrate the presence of anti-ferments in the immune sera in the few tests that were made. It was found that normal as well as immune sera have a marked inhibiting action upon the enzymes of the filtrates, but an accurate study of the differences between the normal and the immune serum was not made. One-tenth of one cubic centimetre of both normal and immune serum prevents the coagulation of 10 cubic centimetres of milk by 1 cubic centimetre of filtrate, and the peptonization of gelatin by 1 cubic centimetre of filtrate.

CONSIDERATION OF SPECIES OF BACILLUS ISOLATED.—We have not succeeded in positively identifying the bacillus. It, however, resembles in most of its cultural characteristics the bacillus described by Sanarelli, the *Bacillus hydrophilus fuscus*, and also corresponds to Russell's description of this bacillus.

Our bacillus corresponds more closely to Sanarelli's bacillus than to Ernst's *Bacillus ranicida*. It does not develop pigment on agar, except for a yellowish tint which appears in older cultures in varying intensity, and thus differs from the bacillus as described by Sanarelli and also by Russell. Both of these observers state that their bacillus is pathogenic for rabbits; our organism, however, has only slight pathogenic properties upon these animals.

The variations noted above are, however, insufficient to warrant us in defining a new species and for the following reasons: the bacillus when first isolated by us produced a small amount of gas, whereas later subcultures fermented sugars without gas production. Similarly the pigment production in the early subcultures was more marked than in the later subcultures. The pigment formation, indeed, was so variable that no constant factors controlling its occurrence were determinable.

When first isolated the cultures of 1904 corresponded closely to the initial cultures taken in the fall of 1903, in respect to the pigment formation on potato, the marked enzymic action upon gelatin, milk, and coagulated serum, and the fermentation of sugars and gas production. Unfortunately, we have not been able to determine the loss of gas formation in later cultures of the bacillus of the epidemic of 1904, as was found to be the case in the previous year.

We have not been able as yet to determine, by means of the reaction of agglutination, the group to which our bacillus belongs. A strong anti-serum for *Bacillus prodigiosus* (1-60,000) fails to agglutinate our bacillus in any dilution, so that we may possibly be justified in excluding it from this group. In determining the group and species characters of a given micro-organism, it is, however, questionable how far cultural features and agglutinability should be taken into account. Thus among the prodigiosus group, races which closely resemble each other in cultural characteristics do not possess similar agglutinative affinities, and an anti-serum developed for one species fails to agglutinate other species which closely resemble the first.

CONCLUSIONS.

The epidemics we have observed were due to the presence and growth in the frogs of *Bacillus hydrophilus fuscus*. This was proved by recovering the bacillus in pure culture from the body fluids of frogs sick or dead of the disease, and the inoculation of healthy frogs with an emulsion of the pure culture, and by obtaining the same clinical picture and pathological findings as in the original diseased frogs; and, finally, by recovering the bacillus in pure culture from frogs inoculated and sick or dying as a result of the inoculation.

The disease is widely distributed throughout North America and Europe, and in this country and Canada is known as "red-leg."

It has been observed by us chiefly in the warm weather of September and October.

The disease is characterized by congestion of the ventral surfaces of the body, with more or less ulceration in, and hæmorrhage beneath, the skin, bloating due to serous exudation into the lymph sacs, gradual failure to respond to stimuli, which symptoms are followed by coma and death, the last being occasionally preceded by tetanic seizures.

After death hæmorrhages into the muscles and degenerative changes in the muscles, spleen, liver, and, to a slight degree, in the intestinal tract, are found. The blood shows an advanced degree of anæmia and leucocytosis.

Predisposing causes of the disease are lesions of the skin, which seem to be the usual portal of entry of the infection, and lowered resistance from heat and from anæmia.

By a series of controlled experiments with inoculated frogs we have shown that, while temperatures a little above freezing have no harmful effect upon the frogs, they completely control all manifestations of the disease in inoculated or diseased frogs, if the frogs are left in the cold for a period as long as seven days; and, further, that even short periods in the cold chamber will bring about a delay of the fatal results in diseased or inoculated frogs.

The anæmia so often found in apparently healthy frogs seems in many cases to be due to the presence in the lungs of the frog of a parasite, the *Distomum cylindraceum*, which, occurring in sufficiently large numbers in an individual frog, is capable of materially diminishing the available supply of red corpuscles.

Severe laking of the blood, the presence of numerous isolated red-cell nuclei, and great diminution in the number, or almost total absence of the red cells in the diseased frogs, are in proportion to the severity of the infection and due to bacterial action.

The presence of the hæmatozoan parasite, the *Drepanidium*, does not play any part as a predisposing or exciting cause of the disease.

The ascarid *Rhabdomena nigrovenosum*, although frequently present as a parasite in the lungs of the frogs, plays no part in causing or promoting the disease.

PRECAUTIONS TO BE OBSERVED IN PREVENTING OR CHECKING
EPIDEMICS IN LABORATORIES.

If frogs are to be kept for more than a few days, care should be taken to avoid supplies from those marshes where the disease is known to prevail year after year, hence the Delaware and Schuylkill valleys which show the disease in an endemic form had best be avoided.

Frogs should not be caught by barrel-staving or any other violent method which may cause abrasions of the skin. The use of a soft, fine-meshed hand-net is to be preferred to other methods.

Before shipment all injured or very pale or thin frogs, or any showing a suspicion of redness of belly or legs, should be put aside, and the rest washed for twenty-four hours in a full stream of cold water.

Shipment should be made in planed wooden boxes, free from projecting knots, nails, or rough surfaces inside. In warm weather ice in some metal vessel placed in the box is to be recommended for delaying over-heating in transit for short distances.

When received, the frogs should be washed thoroughly in an abundance of clean cold water, and should then be kept in a cellar where the temperature in winter is a little above freezing and in summer about 15° C.

The ideal tank is one with smooth sides and bottom and supplied with fresh cold water. The following description of a tank sent to us by Professor George T. Kemp, of the University of Illinois, is so complete that we take the liberty of quoting from his letter:

"The tank consists of an earthenware sink, 3 x 2 feet and 6 inches deep, lined with a hard glaze. It stands on a frame giving it such a tilt that one-third of the bottom is dry when there is 2 inches of water at the lower end. There is a hole in the lower end fitted with a cork, through which runs a glass tube $\frac{3}{8}$ of an inch in diameter, extending into the sink 2 inches from the bottom. The tank is covered by an ordinary window-sash, in which one pane is replaced by a galvanized wire gauze. Water

trickles into the sink from a pipe opening above the gauze and runs out through the glass tube in the cork in the sink-hole. The frogs can shift from wet to dry as they please. The whole sink can be easily disinfected by using sodium hypochlorite with a scrubbing brush. One hundred frogs can be kept in such a tank."

Treatment of the disease when it occurs may be summed up in the words cleanliness, water, and cold.

If the disease appears among frogs in a tank, those with any lesions, such as ulcerations or hemorrhages, or even faint flushing of the belly or legs, should be put into a separate cleaned tank and kept as near 0° C. as possible.

The rest should be removed from their tank to a clean one after being freely washed with an abundance of cold water; the infected tank should be first scrubbed with soap and water, then with a 1-20 carbolic acid solution, and finally flushed out with fresh water. Prof. Colin C. Stewart, of Dartmouth College, uses inclined tanks in series, one water supply sufficing for all the tanks, the diseased frogs being put in the lowest tank as soon as they have been discovered in any of the upper ones. He finds that this method of weeding out the sick ones, together with one thorough cleansing of all the tanks, will check an epidemic, although those already infected may die.

The use of low temperature will in our experience save almost all of those already diseased.

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A METHOD FOR OBTAINING MASS CULTURES OF BACTERIA FOR INOCULATION AND FOR AGGLUTINATION TESTS:

WITH SPECIAL REFERENCE TO PNEUMOCOCCI AND STREPTOCOCCI.

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PLATE XI.

It is often desirable to obtain bacterial cultures in mass for chemical analysis, for purposes of inoculation, or for use in agglutination tests.

In the case of many organisms, pathogenic as well as non-pathogenic, the usual nutrient agar and broth media of our laboratories furnish, under proper temperatures and life conditions, all requisites for abundant growth, and these organisms may thus readily be obtained in mass. On the other hand, certain pathogenic bacteria grow poorly or after slight initial growth die off in the usual artificial media, and it is with much difficulty that these can be obtained in sufficient quantity for chemical study or inoculation, or in the proper conditions for studies in agglutination. This is often due either to a lack of the proper nutriment, or, if this be present, to the accumulation of products of metabolism (principally acids) which either inhibit further growth or actually bring about the death of the organisms.

Often one of the prerequisites of the abundant growth of organisms in our artificial media, either with or without free access of oxygen, is the presence of some carbohydrate or allied substance available in their metabolic processes; and all bacteriologists are familiar with the differences in amount and rapidity of growth of certain organisms in media containing available sugars as compared with the growth in the same media when such sugars are absent. When, however, the acid given rise to by the action of the organisms on the sugar reaches a certain percentage, varying in the case of different organisms, cessation of

growth occurs, and even the death of the organisms may result. With certain sensitive organisms, such as the pneumococcus, the amount of sugar often normally present in ordinary meat infusion broth is sufficient to lead to the death of the organisms after a few days, from the acid produced. On the other hand, the pneumococcus will develop only slightly or not at all in perfectly sugar-free broth.

In the study of fermentations from the time of Pasteur on, it has been known that if certain non-soluble salts, particularly calcium carbonate, not positively influencing the alkalinity of the medium, be added to solutions in which fermentations are taking place, the organic acid produced by the action of the organisms on the sugars present would unite with the calcium, liberating carbon dioxide, and be neutralized, and that so long as the other requisite nutrient materials were present in sufficient amount the organisms would continue to ferment the remaining sugar. Whether there is during this period of activity a great increase in the actual number of bacterial cells depends, however, often on other factors than the mere viability and fermentative activity of the organisms concerned, as, for instance, upon the amount of free oxygen furnished the organisms during this period, and doubtless upon other nutrient conditions not so well understood.

Although this scheme of neutralization of acids formed from the sugars normally present in ordinary media has been taken advantage of by various workers, and an addition of calcium carbonate has been recommended for the removal of acid and the preservation of the viability of organisms sensitive to even small amounts of acid [in case of the pneumococcus (*Wurtz and Mosny*); the neutralization of primary acid in the case of diphtheria broth for toxin production], no attention, so far as the writer at present knows, has been given to the use of fluid media, such as meat infusion broth, to which sugar and calcium carbonate have been added, for the purpose of getting these organisms in large amounts and in sufficiently dense emulsions to be useful for large inoculations and in agglutination experiments.

Certain experiments carried on by the writer during the last

four or five years with the above and other objects in view, not pertinent to this communication, have fully demonstrated that a massive growth may be obtained and the life and active growth of even such sensitive organisms as pneumococci and streptococci may be preserved over long periods in fluid media plus sugars, by the application of this principle of neutralization. The pneumococcus, for instance, may be preserved alive and growing at 37° C. in media rich in sugar (4 % or 5 %) from which it continuously produces acid, for long periods even extending over months. In a comparatively short time the density of the growth is astonishing to one familiar with the scanty growth of the organism in the same media under the usual conditions. It is obvious that the employment of such technique may also be useful in determining some of the factors influencing the growth or cessation of growth and factors leading to the death of organisms in certain nutrient media. Such a method may further be useful in other fields of work not germane to the present communication, but of which it is the writer's intention to deal subsequently in another paper.

The writer's chief object in making this short and outline presentation of the subject at this time is the urgency of the need of a satisfactory method of obtaining suitable cultures of sensitive and poorly growing organisms, especially pneumococci and streptococci, for purposes of inoculation and for agglutination tests.

Agglutination tests with pneumococci and streptococci, when the usual broth cultures (either with or without sugars added) or emulsions from agar are employed, have in the experience of most workers been found not only technically unsatisfactory but have given varying and often contradictory results.

On the other hand, the technique recommended by Wadsworth¹ is valuable and reliable, but when a long series of organisms, as in comparative studies, is being tested against the same or various sera, much time and energy must be consumed, when following his technique, in centrifuging and preparing the organisms for the tests.

The method here proposed, which has given eminently satis-

¹ Wadsworth, *Journal of Medical Research*, 1903, x, 228.

factory and striking results in a comparative study of pneumococci and streptococci in immune sera,—results and appearances which are entirely comparable to those familiar in typhoid, dysentery, and various other agglutinations,—is simple and particularly available for the study of pneumococci and streptococci and other organisms which ferment carbohydrates, but which thrive poorly or die out rapidly in the usual media. The streptococci often, as is well known, grow in broth, either with or without sugar, in flocculi or thickly matted masses entirely useless for agglutination purposes. By the proposed method usually a fair and often a good and satisfactory emulsion may be obtained, from which agglutinative limits may easily be determined.

The medium used should be a one or two per cent peptone broth made from meat infusion, which has been brought to neutrality before boiling and coagulation. After filtration for clearing, one per cent of dextrose (or other sugar fermentable by the given organism) and one per cent of calcium carbonate are added. If the medium be acid, the latter salt will of course bring it to neutral. The calcium carbonate may then be well distributed throughout the broth by shaking and the emulsion rapidly decanted into tubes or preferably small Erlenmeyer or Florence flasks, say 100 cubic centimeters to 150 cubic centimeters in each. These are sterilized on three consecutive days at 100° C. in the usual manner. The flasks after inoculation are placed at 37° C. and are thoroughly shaken once or twice a day to neutralize the acid formed and to break up the chains and masses in the case of streptococci. The growth may be sufficient for purposes of agglutination in two days or even in one day, but as a routine up to the present time we have employed a three or four days' growth, which seems to give more uniform results and more marked agglutination.¹ About an hour before using for agglutination tests the culture should be thoroughly shaken and the calcium carbonate and larger clumps, if present, allowed to settle during this time. The sample to be tested should then be taken from the upper portion of the fluid; or the cultures, after

¹ Such cultures may then be preserved in the ice-box. We have tested them frequently in the same sera from day to day, and have found little or no change in their limit of agglutination even after weeks.

shaking, may be centrifugated for a few minutes. This centrifugalization, however, is not necessary if one remembers that a slight primary deposit may occur which is not due to agglutination.

Such emulsions, tested in the serum of pneumonia patients and in the serum of immune animals, give clumps in every way comparable to those seen in typhoid agglutinations. (See photograph, Plate XI.)

Routine agglutination tests are made by adding one cubic centimeter of the serum dilution to one cubic centimeter of the emulsion in small test-tubes. The tubes are placed at 37° C. for two or three hours—after which time the agglutinations are often practically complete—and then transferred to the ice-box to prevent growth taking place and permit of the further deposition of the clumps of agglutinated organisms, and the final control reading made after eighteen to twenty-four hours.

For routine work on patients' sera an organism should be selected which shows little or no tendency to agglutinate spontaneously or upon the addition of normal salt solution in the control. Spontaneously agglutinating cultures of course are met with among the pneumococci, and are frequent with streptococci, but even with these the limits of agglutination can be determined with much certainty if careful comparison with the control is made.

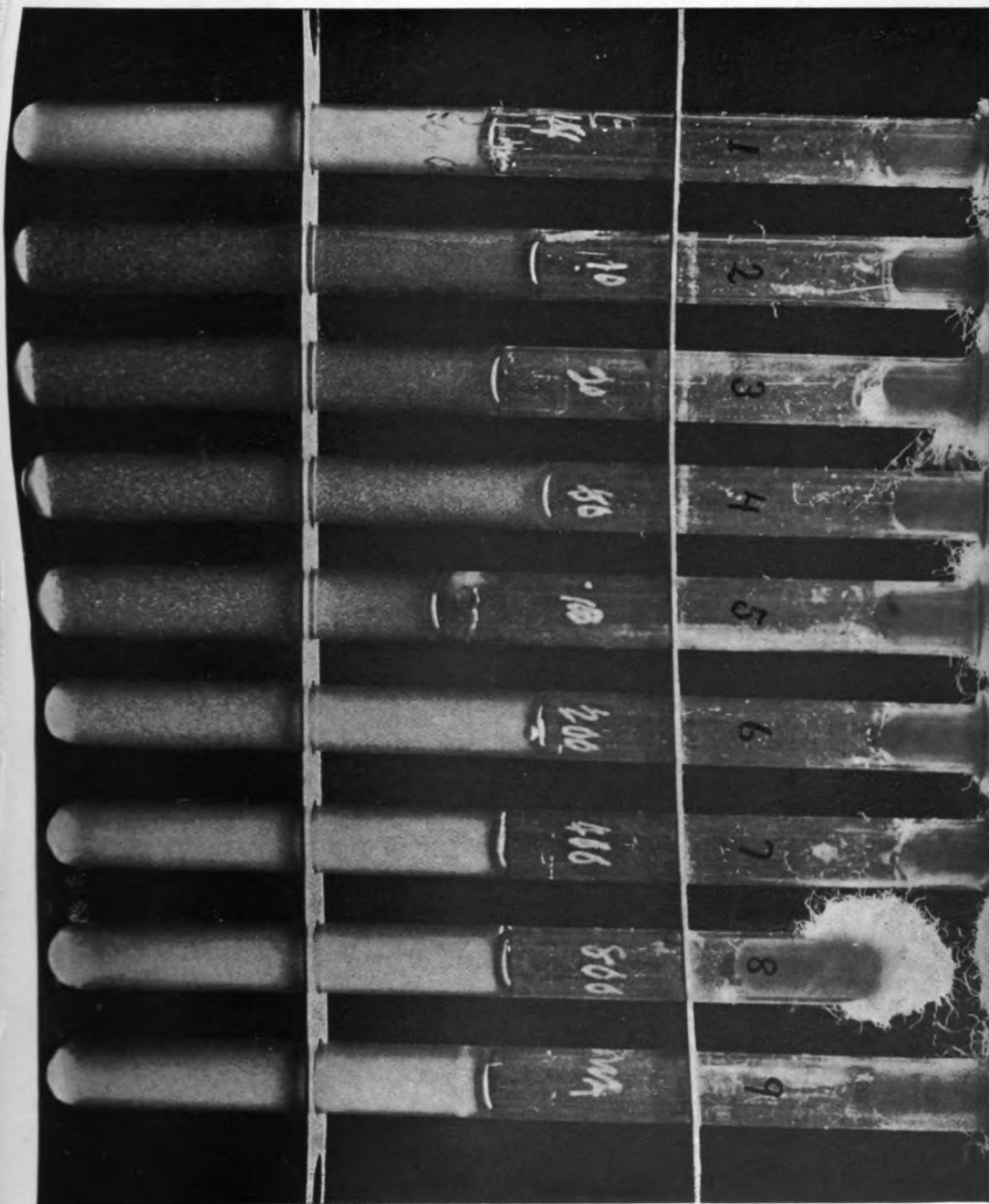
DESCRIPTION OF PHOTOGRAPH.

PLATE XI.

AGGLUTINATION OF PNEUMOCOCCI, GROWN IN CALCIUM-CARBONATE-GLUCOSE BROTH, IN PNEUMOCOCCUS IMMUNE RABBIT SERUM.

The photograph represents the stage of agglutination two hours after making the mixtures, the tubes having been in the incubator at 37° O. during this time. Tube No. 1 contains a 1-20 control of the pneumococcus culture and normal rabbit serum. Tubes Nos. 2, 3, 4, 5, 6, 7, and 8 contain the pneumococcus culture and immune serum in dilutions of 1-10, 1-20, 1-50, 1-100, 1-200, 1-400, and 1-800, respectively, as indicated on the tubes. Tube No. 9 contains a control of normal salt solution and culture. The 1-10 dilution is nearly clear, and large clumps are apparent even in the photograph at 1-200. A hand-lens shows fair clumps at 1-400, and traces of agglutination at 1-800. The controls are uniformly clouded.

Each tube contains two cubic centimeters, one cubic centimeter of the culture and one of the serum dilution, which is made up with normal salt solution. The culture used in the test was grown for four days at 37° O.



THE VIABILITY OF THE PNEUMOCOCCUS AFTER DRYING: A STUDY OF ONE OF THE FACTORS IN PNEUMONIC INFECTION.

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(A Study from the Department of Pathology, Columbia University, under a Grant from the Commission for the Investigation of Acute Respiratory Diseases, of the Department of Health of the City of New York.)

The exact way in which the pneumococcus reaches the lungs of persons suffering from pneumonia due to that organism is not yet thoroughly understood. A number of possibilities have been considered which may be indicated as follows:

(1) The first is that the pneumococcus is frequently present in the saliva, and that when the resistance of a person carrying these organisms is reduced, for example by exposure or overwork, an infection of the lungs takes place either by extension along the tracheal mucosa or by the direct aspiration into the lung of particles of the salivary secretion carrying the germs with them.¹

(2) A second possibility which may be considered is that the pneumococcus is transferred from the oral or pharyngeal mucosa to the lungs by the lymphatics or through the blood.

(3) Another possibility is that the pneumococcus, which is capable of living in masses of dry sputum for some time, is distributed in the form of dust derived from the dried sputum particles and that these particles are inhaled, thus giving rise to a pulmonary infection.

(4) A fourth suggestion is that the pneumococcus is carried

¹ For phases of this problem which cannot be considered here, see Wadsworth, *American Jour. of the Med. Sciences*, 1904, cxxvii, 851. Other papers on the subject are: Nenninger, *Zeit. f. Hyg.*, 1901, xxxviii, 94; Klipstein, *Zeit. f. klin. Med.*, 1898, xxxiv, 191; Dürck, *Deutsches Arch. f. klin. Med.*, 1897, lvi, 368; Wandel, *ibid.*, 1903, lxxviii, 1.

directly from person to person either by the transfer of the normal nasal or salivary fluids, which may contain pneumococci, by coughing or sneezing, or by the spraying of fine particles derived from the sputum of those suffering from pneumonia or other acute inflammations of the air passages, by the same mechanical processes, and that the spray particles thus formed carry virulent organisms to the lungs.

The present study is devoted to a consideration of the possibilities of the aërial transmission of the pneumococcus either in the form of sprayed particles or as dust derived from dried sputum, the modes of infection from the saliva as given in the first and second paragraphs not coming within the scope of this investigation.

While a good deal of work has been done by Cornet, Flügge, and others in determining the viability of the tubercle bacillus and other organisms in fine spray and also after drying and subjection to various physical agents, but little attention has been directed to the pneumococcus except when dried in relatively large masses of sputum. Most observers have considered the pneumococcus as an organism incapable of living for any considerable time when suspended in the form of fine spray.

As the results of recent studies ² on the biology of the pneumococcus have rendered the identification of that organism relatively easy, and as some of the earlier studies on the viability were carried out with bacteria which may or may not have been the pneumococcus, it seemed to the writer that a revision and extension of some of the older investigations might be of value in deciding some points in the mode of transmission of this organism which, though important, have not yet been cleared up.

HISTORICAL RÉSUMÉ.

Before proceeding to the description of the methods and results of personal experiments, it may be well to give a short résumé of the work done by other observers on the general question of transmissibility of the pneumococcus from infectious material to human beings.

² Hiss, *Jour. of Exper. Med.*, 1905 vi, 317.

Viability of Pneumococci in Dried Sputum.—The earlier experiments to determine the dangers of air infection by the pneumococcus were conducted with the idea of fixing the length of time during which the organism would remain virulent for rabbits or mice after drying sputum in bulk, the powdering and diffusion of the powder by air currents being thought to be the means of transmission.

It was known that the pneumococcus died very rapidly in many of the ordinary culture media. In fact it was pointed out by Kruse and Pansini ³ that some varieties of bouillon made from meat infusion were highly bactericidal to the pneumococcus. The same observers found that in body fluids, however, for example in sealed tubes containing pleuritic exudate, the pneumococcus may remain alive for more than a year, if kept in a cool, dark place. They showed that in moist sputum preserved at 15° C. the life of the organism is very short, usually but three or four days. In sputum kept within a few degrees of 0° C., however, the life of the organism is much longer, and while the fluid loses in a few days much of its virulence for mice, yet living pneumococci can be demonstrated for at least six weeks under these conditions.

Drying of the sputum in the air at incubator temperatures killed the organisms quickly, though Guarnieri ⁴ found that rapid drying in a desiccator at 37° C. preserved the virulence for rabbits for four months.

Patella ⁵ noted that rapid drying over sulphuric acid at 16° C. or 38° C. killed the organisms promptly, while slow drying at low temperatures enabled them to live for some time. As it has been shown by Kirstein ⁶ that sulphuric acid probably gives off a small quantity of sulphur trioxide, which would act destructively upon any organisms with which it might come into contact, the rapid death frequently observed when the pneumococcus is desiccated over this medium may be due to the bactericidal

³ *Zeit. f. Hyg.*, 1892, xi, 279.

⁴ *Atti della R. Accad. med. di Roma*, 1888, iv, 97.

⁵ *Ibid.*, 447.

⁶ *Zeit. f. Hyg.*, 1902, xxxix, 166.

action of the acid. Drying over calcium chloride and phosphoric anhydride does not destroy bacteria so quickly as drying over sulphuric acid.

Foà and Bordoni-Uffreduzzi⁷ dried rabbit's blood containing pneumococci on watch glasses and found that the micro-organisms were alive and virulent after forty-five days. Agar tubes inoculated from organs and kept for sixty days showed an abundant growth when placed in the incubator—an evidence that the organisms may remain alive under suitable conditions. At the time at which this work was done, however, the difference between the pneumococcus and the meningococcus had not been thoroughly defined, and the writers termed the organism with which they worked a meningococcus because it was obtained from a case of cerebrospinal meningitis. From their description of its biological features, however, the organism seems to have been *Diplococcus pneumoniae*. It was positive to Gram, showed a capsule, and killed rabbits promptly. Apparently without recognizing their nature, the writers figure pneumococci inclosed in the phagocytic cells of the pneumonic exudate of the rabbit.

Five years later Bordoni-Uffreduzzi⁸ reported the results obtained by contaminating pieces of linen with pneumonic sputum. The cloth was allowed to dry at room temperatures. In one specimen exposed to diffuse light the bacteria remained alive for nineteen days, as determined by injection into rabbits of scrapings from the cloth; in another, fifty-five days. The sputum dried in sunlight was virulent after twelve hours. The results differ somewhat from those of Patella, possibly depending upon the technique, Patella using silk threads soaked in the blood of an animal dying of a pneumococcus septicaemia or threads soaked in broth cultures of the pneumococcus.

The results of Cassedebat⁹ differ considerably from those of Bordoni-Uffreduzzi. The experiments were conducted as follows: Sputum was tested for its virulence and found to kill rabbits. Specimens of this pneumonic sputum were then dried

⁷ *Zeit. f. Hyg.*, 1888, iv, 67.

⁸ *Arch. p. l. sc. med.*, 1891, xv, 341.

⁹ *Revue d'Hygiène*, 1895, xvii, 1066.

on cloth in the air but protected from the direct rays of the sun. Fragments of the cloth were soaked in water and the fluid injected into a rabbit. The results showed that the dried sputum killed rabbits at periods varying from five to twenty-six days, and that fresh sputum from the eighth and ninth days of the disease would not kill rabbits. Apparently the writer relied on the gross post-mortem findings for the identification of the pneumococcus. There is no mention of morphological studies of the blood to identify capsulated organisms or attempts to cultivate the pneumococci. The results have, therefore, but slight value.

Ottolenghi¹⁰ repeated the studies of Bordoni-Uffreduzzi with the following results. The experiments were carried out with three specimens of pneumonic sputum from the fourth or fifth day of the disease. The sputum was spread on linen cloth and allowed to dry in diffuse light at a temperature of 15° C. to 20° C. In explaining the results which he obtained, the author calls attention to the fact that the inoculation of the material into a rabbit is not sufficient to determine whether the pneumococcus is dead or not. The death of the rabbit merely determines the presence of organisms virulent for that animal, but non-virulent forms may be present. He therefore made cultures from the sputum at the same time that he carried out the animal inoculations. The first specimen tested lost its virulence for animals thirty-six days after the preparation was made, whereas pneumococci could be obtained culturally for sixty days after drying. In the second specimen, both methods showed pneumococci at the end of seventy days, and from the third specimen pneumococci were isolated on the eighty-third day. On the basis of these experiments, the author considered that *Diplococcus lanceolatus* can retain its virulence in dried sputum for at least twenty days, and that it remains alive for a considerable time after the virulence has disappeared. The virulence persisted longest in a thin, frothy sputum.

In some experiments recently reported by Heim,¹¹ the viability

¹⁰ *Cent. f. Bakt.*, 1899, xxv, Abt i, 120.

¹¹ *Zeit. f. Hyg.*, 1905, l, 123.

of the pneumococcus after drying was much greater than has usually been assumed. Silk threads were dipped into the heart's blood of cats, rabbits, and mice, which had been killed by injections of pneumococci. The threads were dried in a desiccator over calcium chloride, and were then removed at various periods, placed in bouillon or agar, and the resulting culture inoculated into mice. The organisms were virulent in some cases after 487 days. Great variations, however, were observed. Some of the cultures from the threads no longer gave rise to septicaemia after sixty-six days. One case was not virulent after nine days. In empyema pus the pneumococcus remained virulent for 377 days. Another specimen of empyema pus contained organisms which Heim states lie between the pneumococcus and the streptococcus groups. These were virulent at the end of 149 days and contained viable organisms at the end of 383 days. The conditions of these experiments are, however, highly artificial and cannot be considered as applying very definitely to the question of aërial infections. The alternate drying and moistening of the organisms due to the varying amounts of moisture in the air of rooms is very important as determining the rapid death of the pneumococcus, while protection from such changes by sealing the substances carrying the bacteria in vessels containing calcium chloride tends to prolong the life of the parasite.

Mode of Distribution of Dried Sputum Particles.—It thus having been conclusively shown that the pneumococcus can remain alive for a considerable length of time in dried sputum, it is necessary to demonstrate that this dried sputum, which under ordinary conditions is firmly adherent to the substance on which it is dried, can in some way be reduced to a powder and thus inhaled. Such conditions can only be realized when the sputum is dried in handkerchiefs, bedding, or clothing, and the contaminated material handled, or when the sputum is deposited upon the floor and pulverized by persons walking over the infected area, or distributed in the air by dry sweeping of the floor, or brushing of infected clothes, etc. This mode of distribution of infectious material has been studied chiefly in connection with the tubercle bacillus, because of the ease of identifying that organism in the

infectious dust, and the difficulty attendant upon the recognition of the pneumococcus under the same conditions. The results obtained, however, can be legitimately transferred to the pneumococcus, leaving out of the question for the moment the viability of the latter organism after drying. Much of our knowledge on this subject we owe to the studies of Cornet and of Flügge and his pupils.

Some of the results which have been obtained are as follows: Cornet,¹² who considers this dust inhalation the most important means of infection in tuberculosis, has demonstrated the infectious nature of the dust of rooms in which persons suffering from tuberculosis had lived, and showed that the risk of infection depended very largely upon the expectoration of the sputum on handkerchiefs, bedding, carpets, or clothing, and the subsequent drying of the fluid. He considered that there was practically no danger of direct infection in tuberculosis by particles of sputum expelled by coughing, but that the sputum expectorated in large masses and dried on the bedding or floor was the chief source of the disease. His results have been disputed by Fränkel and also by Flügge and his pupils, who have shown that it is difficult to pulverize the sputum to a sufficient degree to produce a powder fine enough to be carried by air currents of moderate velocity or to remain long in suspension.

Sticher,¹³ in order to test this, pulverized dried tuberculous sputum and found that while the particles could be carried by a current of air with a velocity of 1 cm. per second to a height of one meter, yet the number of bacteria which even the very fine dust particles carried was small, and hence infection was not likely to result from the dissemination of such dust. The air currents in rooms without special ventilation rarely exceed one centimeter per second and do not transport for any length of time coarse dust such as is produced by powdering sputum.

Beninde¹⁴ repeated the experiments of Sticher and found that

¹² *Die Tuberculose, Nothnagel's Spec. Path. u. Therap.*, Bd. xiv, p. 209. Vienna, 1899.

¹³ *Zeit. f. Hyg.*, 1899, xxx, 163.

¹⁴ *Ibid.*, 193.

using handkerchiefs contaminated with tuberculous sputum it was impossible while the latter was still damp to remove any bacilli from the surface of the cloth by a stream of air with a velocity of 10 cm. per second, this being the upper limit of air currents in well ventilated rooms. Tubercle bacilli, however, could be removed by using an air stream of 1 cm. per second, but only after the handkerchief had been carried about for two days and was thoroughly dry.

Further investigations in this line were made by Neisser,¹⁵ who used pneumonic sputum and studied the conditions obtained by mixing the fluid with dust and drying. This combination was then finely pulverized and carried from one chamber to another by an air current with a speed of from 2.8 mm. per second to 23 cm. per second. As a control, some of the sputum used was injected into mice and shown to be virulent. As soon as pneumonic sputum and the dust mixture dried, it would no longer kill mice, therefore the danger of dust infection by inhaling pulverized and dried sputum seemed exceedingly remote.

It should be repeated, however, that while it is not difficult to obtain dry and finely pulverized pneumonic sputum under experimental conditions, yet practically the drying of the mass is rarely complete enough to permit thorough powdering, and the particles which are removed mechanically from the sputum, after this fluid has dried on cloth, wood, or metal, are of such dimensions that they cannot be carried for any considerable distance by the air currents ordinarily found in well ventilated rooms, or, if so carried, remain in suspension for a very short time. The contaminated particles are not likely, therefore, to be inhaled even by persons in close contact with the patient, and are still more unlikely to lead to an infection of persons in other rooms or at a distance. Only in structures subject to strong draughts, such as factories or railroad carriages, are air currents likely to be strong enough to render these coarse particles dangerous. The possibilities of infection are reduced to a minimum when the dust particles are blown about in the open air. The dilution is so great and the death of the organisms

¹⁵ *Zeit. f. Hyg.*, 1898, xxvii, 175.

contained in the dust is so rapid that infection cannot be assumed as likely to occur.

Germano¹⁶ studied the effect of drying the pneumococcus with dust by a different technique. The results which he obtained with cultures of the pneumococcus showed that when mixed with sterile dust and dried, the organisms died within two days, unless the drying took place at a temperature below 0° C., when the organisms remained virulent for eight days. Mixed with sand and vegetable soil, the organisms died in two days; mixed with volcanic ash (Tuffboden), and kept moist, the organisms remained alive for six days.

Another group of experiments with another organism, presumably pneumococcus, showed a very considerable variation from the above. The culture mixed with brick dust remained infectious for forty days, either kept moist, dried in the air, or dried over sulphuric acid. Mixed with sand this same organism was infectious for sixty days when kept moist or dried at room temperature, and for fifty days when dried over sulphuric acid. This organism was obtained during an epidemic of pneumonia which occurred in a small village, a number of cases developing about the same time. Germano thinks that possibly the epidemic was due to the long life of this organism in the air.

The results of experiments by the same writer with pneumonic sputum confirmed the facts which have been observed as to the long life of the bacteria when dried in coarse particles. For example, pneumonic sputum mixed with room dust kept moist was virulent for twelve days, kept dry, for twenty days, dried over sulphuric acid, for sixteen days, dried at a low temperature, for eight days. The last results are thus somewhat different from those obtained by Patella and Neisser. Sputum mixed with earth (Humusboden) was virulent for twelve days when kept moist, for one hundred and forty days when dried in the air, and for one hundred days when dried over sulphuric acid. With a low temperature the virulence was retained for only sixteen days.

The writer considers that slight variations in the type of the

¹⁶ *Zeit f. Hyg.*, 1897, xxv, 439; *ibid.*, 1897, xxvi, 66 and 273.

diplococcus may contribute very largely to the length of time during which the organisms can resist drying. It was certainly proven that they remained virulent longer when dried than when kept moist. At low temperatures the short life of the organisms seemed to be conditioned by the fact that the sputum dried slowly at a point near 0° C. so that the bacteria were really kept moist. The rapidity of the drying process at room temperature had no influence upon the life of the diplococcus. Germano concludes, finally, that air infection of human beings by the organism is possible with the pneumococcus, but the chances are relatively small.

It will be seen from the preceding résumé that the views expressed by Cornet on the possibility of dust infection in tuberculosis can hardly be considered as of great import in pneumonia. The sputum of pneumonic patients is often exceedingly viscid and thick; it is not usually produced in the same abundance as in pulmonary tuberculosis; and the coughing of the patients is rarely so prolonged or strongly expulsive. Coarse particles are therefore less likely to be distributed in the neighborhood of such a patient. When such particles are expelled, the results of Sticher and Beninde show that the air currents which are ordinarily present in well ventilated houses are insufficient to remove bacteria from the moist or dried sputum. The risk of infection must be largely confined to those who handle the bedding, etc., of pneumonic patients.¹⁷ The experiments of Neisser and Germano are not wholly consistent, but tend to show that the pneumococcus dies early when dried in finely divided sputum. It is possible that in some of Germano's experiments the organism was not the one which we are accustomed to consider as the pneumococcus.

Conditions under which the Pneumococcus may be Transmitted in Sprayed Particles.—We are largely indebted to Flügge and his co-workers for the experimental investigation of the theory that the transfer of pathogenic bacteria from one person to another is

¹⁷ See in this connection a report by Edson and Ghiskey on "A Hospital Epidemic of Pneumococcus Infections," *Trans. of the Philadelphia Coll. of Phys.*, 1904, xxvi, 6.

possible by the aërial transmission of fine spray particles. They considered that the spraying of fine particles of sputum of saliva by talking, coughing, sneezing, or spitting, might carry infectious material from one person to another if the specific organism remains alive in such spray for a sufficient time to permit the floating particles to be carried from the patient to other persons in the vicinity by the air currents produced for purposes of ventilation.

The studies of this means of transmission of the pneumococcus are not numerous, and we have obtained a large part of our knowledge concerning such transmission by investigating the conditions of infection which obtain in connection with pulmonary tuberculosis. Here again most of the factors are the same with both the pneumococcus and the tubercle bacillus, and we can without impropriety transpose the results obtained by the study of one organism to another.

While it has been repeatedly shown that the air expired from the lungs during quiet breathing contains no bacteria, yet in disease a small number of bacteria may be given off. It has been shown by Koelzer¹⁸ that tuberculous persons by a sort of internal spraying give off tubercle bacilli even during quiet breathing, and that the fine drops containing tubercle bacilli are produced by the pulverizing of the thick mucus in the bronchi during the passage of the expiratory current—the same physical condition which gives rise to the râles heard on auscultation of the chest. Laryngeal tuberculosis increases the liability to the contamination of the expired air. Koelzer obtained positive results in one case out of fifteen persons examined, care being taken to see that no coughing took place while the Petri dishes were exposed to the expiratory current. A similar observation is recorded by Schäffer,¹⁹ who was able to demonstrate lepra bacilli in the expired air of persons suffering from lesions of the nose and throat due to that organism.

The results of many observations on the bacterial content of the expired air have shown, however, that this source of infection

¹⁸ *Zeit. f. Hyg.*, 1903, xliv, 217.

¹⁹ *Arch. f. Derm. u. Syphilis*, 1898, xliii and xlv, 159.

may be practically neglected. On the other hand, coughing and especially sneezing, as previously mentioned, cause an abundant spraying of fine fluid particles which may contain bacteria.

Koeniger²⁰ has studied with great care the conditions under which this spraying takes place. Large numbers of particles are produced when the expiratory stream is interrupted and then suddenly begun. The drops are formed in the portion of the respiratory tract where the stoppage of the air current takes place. Particles produced in the larynx are often stopped by the lips so that the method which the person uses in coughing causes great variations in the number of particles expired in the air. If the mouth be kept nearly closed, the laryngeal particles do not escape in large numbers. If, however, the mouth is tightly closed, drops may be produced from the passage of the air currents over the lips, as the latter are pushed open by the cough. Particles are also sprayed out in clearing the throat. Loud speaking gives more spraying than quiet conversation, and the method of articulation exerts considerable influence. The letters k, p, f, and t cause more spraying than vowels or other consonants. It is probable that more germs are sprayed from a thin, watery sputum than from a thick, mucous variety, but the intensity of the coughing impulse is much more important than the consistence of the sputum. More particles are expelled when the cough is short and sharp. Koeniger's studies on spraying were made by infecting the mouth of the experimenter with *B. prodigiosus* and other saprophytic organisms and then exposing large numbers of plates while talking, coughing, or sneezing.

Dimensions of the Sprayed Particles.—The size of the particles produced by coughing varies greatly. Heymann²¹ studied the size and number of the drops by catching the spray produced by coughing on glass slides and measuring the size of the drops so produced. The finer particles had a diameter when flattened on glass of from thirty to forty micra. Sneezing, according to my own observations, may give rise to a very fine spray, the particles not measuring over ten to twenty micra. The same is true of the

²⁰ *Zeit. f. Hyg.*, 1900, xxxiv, 119.

²¹ *Ibid.*, 1899, xxx, 139.

drops produced in a spray apparatus using either a hand bulb or air at high pressure. Many of the particles are very small and contain no bacteria. Those above twenty micra usually contain one or more organisms if the sputum is rich in bacteria. Even the smaller particles, however, though they may not contain bacteria, when collected and examined are found to have a nucleus formed either by mucus or by salt crystals. The evaporation from these small particles when sprayed into the air is exceedingly rapid, because of their small size.²²

Spatial Distribution of the Sprayed Particles.—Flügge in a series of papers²³ has shown that a person with a cough sprays fine particles into the surrounding air, the radius of the zone of such spraying being usually one meter, rarely two meters. Within this area therefore the air may contain floating particles carrying pathogenic bacteria. Flügge has shown that fine dust particles laden with bacteria may be carried horizontally by a stream of 0.2 mm. per second, one five-hundredth of the speed of a barely perceptible draught. Upward translation of these motes requires a slightly greater wind velocity, about 0.3 to 0.4 mm. per second. Stronger currents of air may carry them to great distances. Hutchinson²⁴ was able to demonstrate the transportation of particles containing *B. prodigiosus* for a distance of 600 meters. The drops produced by coughing, sneezing, etc., are usually larger and heavier than those just mentioned, and Heymann²⁵ has shown that a large proportion of them settle out of the air of an ordinarily ventilated room within an hour. Particles of this size are not transported laterally to any very great extent. In fact, it is exceedingly difficult to demonstrate tubercle bacilli in the air of wards containing tuberculous patients.

As the sprayed particles settle they adhere to the furniture, walls, bedding, and carpets, and dry. It is then impossible to remove them by any stream of air within the limits of ordinary

²² See on this point Thomson, *Conduction of Electricity through Gases*, Cambridge, 1903, p. 135.

²³ *Zeit. f. Hyg.*, 1897, xxv, 179; *ibid.*, 1899, xxx, 107; *ibid.*, 1901, xxxviii, 1.

²⁴ *Ibid.*, 1901, xxxvi, 223.

²⁵ *Ibid.*, xxxviii, 21.

ventilation currents. It is possible to remove these particles, however, by dry brushing, sweeping, or dusting, and the powder so formed may float for a long time in the room or be transported to adjacent ones.

Bacterial Content of Sprayed Particles.—Direct evidence of the bacterial content of the sprayed sputum has been obtained by B. Fränkel, who examined the contents of two hundred and nineteen face masks each of which had been worn for twenty-four hours by persons whose sputum had contained tubercle bacilli. In twenty-six of these masks tubercle bacilli could be demonstrated. Fränkel assumes that in thirty-two days 2600 tubercle bacilli had been caught in masks which would otherwise have escaped into the air. In a considerable number of cases of tuberculosis, however, Fränkel pointed out that the masks remained uninfected so that only a small number of patients could be shown to cough out drops of sputum or saliva containing tubercle bacilli.

The presence of virulent organisms in sprayed sputum has been verified by other observers.²⁶ The most detailed study is perhaps that of Heymann,²⁷ who examined with especial care the conditions attending the spraying of tuberculous sputum by patients under, so to speak, natural conditions—that is, the coughing was not forced, the patients simply being confined to a room while the tests were carried out. The particles sprayed out by the patients were collected and found to contain abundant tubercle bacilli.

An interesting example of the large numbers of bacteria which may be expelled is that reported by Schäffer²⁸ where one leprosy patient at a single sneeze gave off 25,000 bacilli, and another patient, 110,000. Patients with severe lesions of the tuberculous type gave off from 10,000 to 185,000 lepra bacilli in ten minutes' talking. The sprayed bacteria were caught on slides placed close to the patients' mouths and only a very few could be demonstrated at a distance of one and one-half meters.

²⁶ v. Hübener, *Zeit. f. Hyg.*, 1898, xxviii, 348; v. Weismayer, *Wiener klin. Woch.*, 1896, p. 1039; Bing, *Zent. f. innere Medizin*, 1905, p. 54; Mendes de Leon, *Arch. f. klin. Chir.*, 1904, lxxii.

²⁷ *Zeit. f. Hyg.*, 1901, xxxviii, 21.

²⁸ *Arch. f. Derm. u. Syphilis*, 1898, xliii and xliv, 159.

Hamilton²⁹ finds that streptococci are expelled from the mouth by coughing or even by breathing by persons with streptococcus infection of the upper air passages.

Life of the Bacteria in the Spray Particles.—In order to study the length of life of bacteria in sprays, Laschtschenko³⁰ atomized diluted pneumonic sputum (ten parts sputum and from one to two parts water) in a closed vessel and caused the particles to be carried upwards for one meter by a current of air of from 6 to 10 mm. per second.

The spraying was continued under low pressure for one and one-half hours. The particles were collected and the fluid injected into mice, with three positive and two negative results. Spraying *undiluted* sputum with air speeds of 10 to 12 mm., he obtained one positive and six negative results, the infectious nature of the sputum being previously determined by injecting mice. Using the same apparatus and conditions with phthysical sputum, a positive result was obtained in all cases with air speeds of from 6 to 14 mm. per second, the sputum being diluted and undiluted. The spray was produced by a very low air-pressure stream. The results show that the pneumococcus and the tubercle bacillus can live for a longer or shorter time in sprayed sputum. The writer gives no explanation of the fact that many more positive results were obtained with tuberculous sputum than with the pneumococcus, but it is evident from my own experiments, to be given later, that the drying which the pneumococci underwent while carried up in the air current was sufficient to kill many of the organisms.

A phenomenon noted by Koeniger³¹ is of interest in this connection. He observed that after spraying large quantities of cultures of *B. prodigiosus* over the floor and furniture of a room it was impossible to obtain colonies of this organism on exposed plates even when large amounts of dust were produced by energetic brushing. Growths were obtained from many other organisms but not from the prodigiosus. This fact was not fully understood by Koeniger, who states that the results of his

²⁹ *Jour. of the American Med. Assoc.*, 1905, p. 1108.

³⁰ *Zeit. f. Hyg.*, 1899, xxx, 133.

³¹ *Ibid.*, 1900, xxxiv, 119

experiments show that the bacteria must be moist to produce a growth. The true explanation was given shortly afterward by Kirstein, who showed that the reason for the negative results was that *B. prodigiosus* when sprayed in fine particles dried rapidly and was promptly killed, especially when exposed to diffuse daylight. In one set of experiments by the latter, *B. prodigiosus* was sprayed in two rooms and the falling germs caught on glass plates. In the dark room the bacteria remained alive for fifteen days, in the well-lighted room for only three days.

Similar results were obtained by Kirstein³² for pathogenic organisms such as the typhoid bacillus, which remained alive for only a few hours, the tubercle bacillus, which was alive for from four to eight days in diffuse light and as long as forty days in the dark.

Staphylococcus pyogenes aureus and *streptococcus* remained alive for from ten to sixteen days; diphtheria bacilli, less than twenty-one hours; anthrax bacilli, nearly ten weeks. The pneumococcus was not investigated, as the author assumed from the results of previous studies by Neisser and others that prompt death of the pneumococcus occurred after drying.

In a more recent paper, Kirstein³³ finds that the tubercle bacillus lives for from eight to fourteen days when sputum is sprayed on fine dust, from four to seven days when tuberculous sputum is finely powdered, five days when deposited on fine cloth fibers, and finally that the bacillus lives but three days on fine street dust although it was alive for eight days on coarse dust of the same variety. In all of these tests the bacteria were exposed to diffuse daylight.

All observers are agreed then that the life of the bacteria when sprayed and dried may be safely assumed to be much shorter than when they are dried in masses. Diffuse light and especially sunlight rapidly destroy the organisms, while preservation in a dark, cool place tends to prolong their existence.

³² *Zeit. f. Hyg.*, 1900, xxxv, 123; *ibid.*, 1902, xxxix. See also Ficker, *Zeit. f. Hyg.*, 1898, xxix, 1.

³³ *Zeit. f. Hyg.*, 1905, l, 186.

Summary.—As will be seen from the survey of the bibliography of the subject just given, the conditions of the viability of the pneumococcus have been fairly well established when either sputum or other fluids containing the organism are dried in bulk and exposed to diffuse daylight or the direct rays of the sun. There are minor inconsistencies in the results dependent upon the method used, the sensitiveness of the animal employed to determine the presence of living pneumococci, and possibly also upon slight variations in resistance of the various strains. The identification of the organism was, however, so far as is reported in many of the studies, entirely dependent upon either the morphology of the bacteria isolated or even upon the death of the animal without any microscopical verification of the presence of a septicæmia. As it has been shown that there are other capsulated organisms which are fatal to mice if given in sufficiently large amounts, and as these animals and also rabbits frequently die after the injection of sputum without the presence of pneumococci being determinable either morphologically or by culture, it seemed to the writer that a few experiments might properly be devoted to a repetition of the studies of the earlier Italian and German workers whose papers have already been considered. The experiments of Germano in mixing cultures of the pneumococcus or sputum with sterile dust were not repeated for they are very complete as they stand and are not especially pertinent to the question in hand.

The main portion of the writer's studies were therefore devoted to the investigation of the question of fine sprayed particles containing pneumococci and the length of life of the organisms in this spray. This ground has not been fully covered by previous workers, and as its great importance in the transmissibility of the tubercle bacillus has been shown, it seemed proper to extend our knowledge to the pneumococcus although it has generally been assumed that that organism was too sensitive to desiccation to live very long in fine particles.

I.—EXPERIMENTS ON THE VIABILITY OF THE PNEUMOCOCCUS IN LARGE MASSES OF SPUTUM.

EXPERIMENT I.—The following tests were made to determine the viability of the pneumococcus in sputum when kept moist and at room temperatures and also when kept at 0° C.

TABLE I.
TESTS WITH MOIST SPUTUM.

Day of Test.		1	5	10	15	20	30	45	60
I. Thick, mucous sputum from 3d day of pneumonia	22° C.	+	+	0	0	0	0	0	0
	0° C.	+	+	+	+	+	+	+	0
II. Thick, rusty sputum from 3d day of disease....	22° C.	+	+	+	0	0	0	0	0
	0° C.	+	+	+	+	+	+	0	0
III. Thin, fluid sputum from 5th day of disease.....	20° C.	+	+	+	+	+	0	0	0
	0° C.	+	+	+	+	+	0	0	0
IV. Thin, yellowish sputum after crisis.....	20° C.	+	+	0	0	0	0	0	0
	0° C.	+	+	+	+	+	+	+	0
V. Thick, yellowish sputum from 8th day of disease...	20° C.	+	+	+	+	0	0	0	0
	0° C.	+	+	+	+	+	+	0	0

The positive marks mean that the pneumococcus was either isolated from the sputum by culture, or, especially after the 5th day, that the subcutaneous injection of from one fourth to one fifth of a cubic centimeter of the undiluted sputum was fatal to a mouse. No result was considered as positive unless capsulated, Gram-positive organisms could be isolated from the blood of the animal, and unless the coccus fermented inulin after plating out on chest-serum agar. Occasionally by the use of very large quantities of sputum (0.5 to 1.5 c.c.) it was possible to kill mice up to fifty days, but often only one animal out of three died, showing that only a few organisms remained alive.

It will be seen from the table that the life of the pneumococcus in fresh, moist sputum at room temperatures is rarely over two weeks. The specimens were kept in the dark in order to compare them directly with those at 0° C., which were of necessity inclosed in a cold-storage box. Two specimens kept in strong diffuse daylight lost virulence for mice in less than five days.

The rapid death of the organisms in sputum as compared to chest-serum is possibly due to the bactericidal action of the mucus of the sputum.

EXPERIMENT II.—Tests were also made by drying sputum in Petri dishes at room temperatures. Some of the specimens were kept in a dark, dry spot, others were exposed to diffuse daylight in a room facing the south, others were exposed to full sunlight. Fragments of the dry crust of sputum were then removed, rubbed up in sterile bouillon, and inoculated into mice. Other specimens

were finely powdered in a mortar with a few fragments of glass, and the dust exposed to daylight or direct sunlight. The results are as follows:

TABLE II.
TESTS WITH DRIED SPUTUM.

Day of Test.	1	4	8	12	20	30	40	60	70	80
Sputum kept in dark:										
No. I. Thin, watery.....	+	+	+	+	+	+	+	o	o	o
" II. Thick, mucous....	+	+	+	+	+	o	o	o	o	o
Sputum exposed to diffuse light:										
No. I. Thin, watery ...	+	+	+	+	+	+	o	o	o	o
" II. Thick, yellow, mucous.....	+	+	+	+	+	o	o	o	o	o
" III. Thick and rusty.	+	+	+	+	+	+	+	o	o	o
Sputum dried over calcium chloride in daylight:										
No. I. Thin, watery.....	+	+	+	+	+	+	+	+	o	o
" II. Thick, mucous ...	+	+	+	+	+	+	+	+	+	o

The specimens of sputum dried over calcium chloride retained their virulence for mice for a slightly longer period than those exposed to the air. This is probably due to the very complete and prompt drying which takes place. The specimens exposed to the air never dry completely, and the amount of moisture retained varies from day to day in accord with the atmospheric changes.

TABLE III.
TESTS WITH DRIED AND PULVERIZED SPUTUM.

Hours of Test.	1	2	4	8	12	24	36	48
Sputum dried and exposed to sunlight:								
No. I.	+	+	+	o	o	o	o	o
" II.	+	o	o	o	o	o	o	o
" III.	+	+	o	o	o	o	o	o
Sputum finely powdered and kept in dark:								
No. I.	+	+	o	o	o	o	o	o
" II.	+	+	+	o	o	o	o	o
Exposed to diffuse light:								
No. I.	+	o	o	o	o	o	o	o
" II.	+	o	o	o	o	o	o	o
Sputum finely powdered and exposed to direct sunlight:								
No. I.	o	o	o	o	o	o	o	o
" II.	o	o	o	o	o	o	o	o

It is evident from the table that the exposure of the pneumococcus to sunlight results in the prompt death of the organism. The mere powdering of the sputum also destroys the pneumococcus, a phenomenon probably due to the rapid and complete drying which takes place. The action of even diffuse daylight in hastening the death of the organism is evident from the table. Exposure of the powder to sunlight effects an even more rapid destruction, there being probably three factors in the process. One is the formation of oxidizing agents, probably hydrogen peroxide, by the action of the sun's rays upon the traces of moisture remaining in the sputum,³⁴ a second the rapid drying which takes place, and a third, the destructive action of the chemical portion of the sun's rays.

EXPERIMENT III.—Sputum was spread on fragments of sterile wood, and tin, and on woollen and cotton cloth. The specimens were allowed to dry, and were kept either in diffuse daylight or sunlight. The life of the organism was about the same on wood and tin as on glass. On cloth several tests gave a slightly longer life, the sputum being virulent for mice after sixty days. This is explained by the penetration of the cloth which takes place when soaked with sputum, the fiber of the cloth protecting the organism from light and the layer of sputum formed being thicker than on a flat surface. This effect was more marked in those fragments exposed to sunlight, one piece of woollen cloth being virulent to mice after twelve hours' exposure, about six hours being given on two successive days in May. The death of the bacteria occurred on two hours' further exposure.

II.—VIABILITY OF THE PNEUMOCOCCUS IN FINE SPRAYED PARTICLES.

Technique.—In order to spray sputum and other fluids containing pathogenic bacteria and to collect the finer particles, it is necessary to conduct the operation in an air-tight chamber, to avoid contamination of the laboratory and infection of the operator.

The apparatus employed by the writer was modelled upon the one described by Kirstein,³⁵ with some slight modifications. The box was constructed of seven-eighths inch white wood lumber with internal measurements of 38 cm. in depth, 35 cm. in width, and 152 cm. in length. At one end were perforations

³⁴ Bie, *Mitth. aus Finsens Med. Lysinstitut*, 1905, Neuntes Heft, p. 5.

³⁵ *Zeit. f. Hyg.*, 1900, xxxv, 145.

for the insertion of the tip of the spraying apparatus and apertures to permit of the escape of air driven into the chamber while spraying the sputum. In order to prevent direct carrying of particles the full length of the chamber and the deposition of the organisms in coarse masses upon the Petri dishes or other substances used to collect the spray, two baffle plates were placed about the middle of the chamber, 22 cm. apart. These plates were of glass and measured 28 by 35 cm. They were held in place by narrow strips of wood nailed on the inner side of the box, and further secured by putty and a layer of enamel paint. The plate nearer the spraying apparatus was so placed that its upper portion was in contact with the lid of the box. The plate farther from the spraying apparatus was in contact with the floor of the box, leaving a space of 10 cm. between its upper edge and the lid (see Fig. 1). It was thus impossible for particles from the spray to pass directly from one end of the box to the other. The coarser masses strike the first plate and adhere to it. Only the finely suspended particles pass over the top of the second plate, and this only when a current of air is drawn through the apparatus (see Experiment IV).

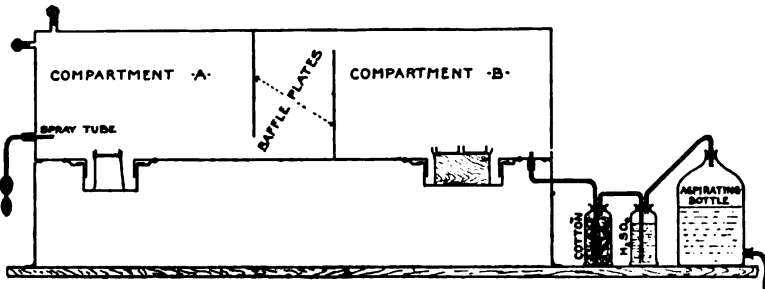


FIG 1 Diagram of Box for Spraying Pathogenic Bacteria.

In order to collect the sprayed particles, three apertures were made at the bottom of the box, two of which were circular, measuring 13 cm. in diameter; the third was rectangular and measured 26 by 14 cm. These openings had a tin collar inserted in them extending about 10 cm. below the bottom of the box. These collars were rendered air-tight by white lead. Each opening was closed during the experiment by placing under it a dish some 3 or 4 cm. larger in diameter than the collar, the dish being filled with 1:1000 mercuric chloride solution, thus making a water seal. The dishes were held in position by the use of small cupboard buttons which could be swung into place under the rim of the dish. Before the spraying was commenced, suitable receptacles for catching the spray, such as Petri dishes, either dry or containing culture media, or fragments of sterilized cloth, wood, tin, etc., were placed on small stands which rested on the bottom of the dish. For the smaller apertures in the first compartment these stands were ordinary drinking-glasses which were inverted and the upper end surrounded by a strip of half-inch surgical adhesive plaster. The Petri dish placed on this adhered quite firmly, and there was no danger of its falling off during the process of removal, even though the glass

ably tipped. In the larger rectangular opening the stand was made of half-inch pine board with four nails for legs, surrounded by a collar of adhesive plaster. This collar retained the plates in position and prevented their shifting during insertion or removal of the stand. The surface of the Petri plate when inserted was approximately level with the bottom of the spraying-box.

In order to render the inside of the box air-tight and waterproof, the corners were filled with putty and the inside was given three coats of thick enamel bath-tub paint. The lid was held in position by one-eighth inch steel wires which passed from a turn buckle fastened to the lid on one side, underneath the box and over a wooden brace to a turn buckle on the opposite side. The turn buckles could be screwed tight, thus holding the lid firmly in position. In order to make a suitable seal, the upper edge of the box was smeared with a thick layer of paste sold commercially as anti-phlogistine. This was found to be better than putty or white lead, as it did not set, but remained moist and somewhat pliable for a period of nearly two months. At the end of the box farthest from the spraying apparatus was an aperture similar to those in the spraying end of the box. A suitable opening was made by boring a hole from the outside of the box, about 22 mm. in diameter. A collar, about 3 mm. in width, made of wood, was left on the inside of this opening. Short pieces of brass tubing were heated over a Bunsen flame, smeared with rosin, and quickly inserted in the holes, into which they fitted snugly, the collar of wood which was left insuring a firm seat. As soon as the rosin cooled, an air-tight joint was obtained. Four of these apertures were made: one for the insertion of the spray tube, one for aspiration of the air current, two for egress of the air forced in by the pressure apparatus. The spray was produced by a long glass spray tube which was inserted through one of the openings and tightly packed in position with absorbent cotton. After the spraying was completed, these tubes could be easily sterilized by boiling in one per cent. sodium carbonate solution. The spraying was done by means of compressed air, the pressures used varying from five pounds or less to the square inch to forty pounds to the square inch. It was found necessary to use a higher pressure in the case of thick, mucous sputum than for thin, watery sputum. By this means suitable quantities of thick sputum, in many instances 30 to 40 c.c., could be atomized during the course of ten minutes. Before beginning the experiments the box was tested by closing all the apertures with corks and inserting a water manometer in one of the openings and forcing in air through another. A pressure of three inches of water was sustained for fifteen minutes, showing that the box was air-tight for this pressure. Higher than this it was impossible to go because the water-seals would have been forced by the pressure. As it was, some difficulty was experienced in spraying into the apparatus unless air was being drawn out at the same time, because of the escape of bubbles of air through the water-seals. It would be advisable, therefore, to modify the apparatus and make the seals somewhat deeper; possibly 15 cm. would be better than the 10 cm. used. Air was drawn through the box by means of an aspirating bottle graduated in liters. In order to catch any bacteria and prevent their entry into the aspirator, a bottle was inserted between the box and the aspirator containing tightly packed absorbent cotton, and a second containing sulphuric acid through which the aspirated air was forced to bubble. The rate of aspiration could be measured

by timing the rate of outflow from the bottle. The speed at which the particles were carried from the chamber nearer the spraying apparatus, which may be for convenience termed A, into the chamber farther from the spraying, called B, could be determined as follows. (Fig. 2.)

The distance between the two baffle plates being 22 cm., their height 28 cm., the length of the hypotenuse would be about 36 cm. The diameter of the channel is then approximately 6 cm. The cubic contents of this channel from the lower aperture of the first baffle plate to the upper aperture of the second would be 36 by 35 by 6 cm. This is approximately 7560 c.cm. If this amount of air is aspirated from the farther end of the box in one minute, the velocity in the channel will be 6 mm. per second, which is near the lowest limit of air speed which will move very fine particles. Air speeds, therefore, were used in these experiments of from 2 to 10 mm. per second.

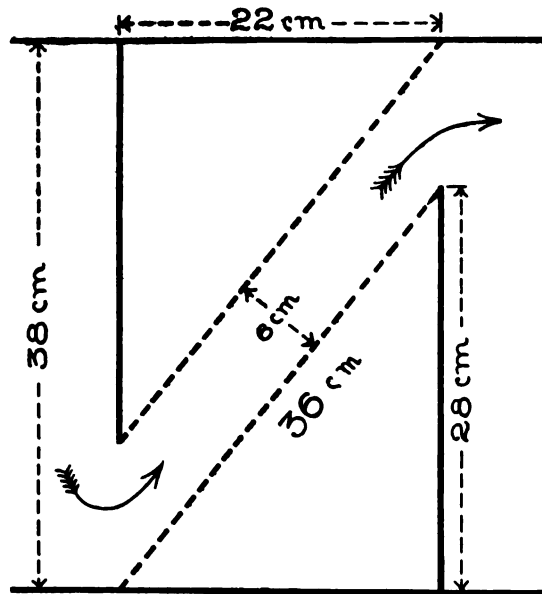


FIG. 2. Diagram of Dimensions and Course of Air Current in Spraying Box.

EXPERIMENT IV.—A preliminary experiment made with bouillon cultures of *B. prodigiosus* showed the box to be tight and that no bacteria passed the baffle plates unless a current of air was drawn through the box.

Sputum was then obtained from a case of acute lobar pneumonia at about the third day of the disease. Morphological examination showed numerous diplococci in nearly pure culture. They were positive to Gram, but no capsules were demonstrable. The sputum was plated on chest-serum agar and diplococci isolated which were Gram positive, had well marked capsules, fermented inulin, and killed a mouse in three days, capsulated cocci being found in the heart's blood.

Ten cubic centimeters of this thick sputum were sprayed in the box, using 40 pounds air pressure. During the spraying and for some time after, a slow current of air was drawn through the apparatus at a rate of about 0.2 mm. per second. Cover-slips exposed in the second compartment during spraying showed numerous particles derived from the spray, some of which contained pneumococci or the other bacteria of the sputum. A current of 0.2 mm. per second is therefore capable of transporting spray-carrying bacteria for a distance of at least one meter.

After the spray had been stopped, covers were exposed every fifteen minutes for two hours. At the end of an hour most of the particles carrying bacteria had settled. Covers exposed after ninety minutes had elapsed showed no bacteria, only very small particles of mucus taking a blue stain with gentian violet. This shows that the bacteria may be assumed to settle 38 cm. in from sixty to ninety minutes.

In order to study the settling of the particles more conveniently and to determine the length of time for which sprayed sputum particles can remain in suspension, ten cubic centimeters of this very thick sputum were sprayed at 40 pounds pressure into a tall aspirating jar about 45 cm. in height, the air contents of which had been cleansed of dust by aspiration through a thick cotton plug. A jar was used for this preliminary experiment instead of the box just described, because of the ease with which suspended particles could be rendered visible by a strong beam of light. A thick fog of the sprayed particles was produced which remained suspended for sixteen hours and could be rendered easily visible by passing a beam of light from an electric arc. At the end of twenty-four hours only a few fine particles could be seen on concentrating the light with a lens. No bacteria were deposited on cover-glasses or culture plates after the jar had stood for two hours.

In order to determine whether the fine spray particles, which remained a long time in suspension after spraying a broth culture, contained bacteria, the jar was filled with spray from a bouillon culture of *B. prodigiosus*, and after standing one hour the plug was removed from the upper end and the bottle was reversed and allowed to rest on the mouth of a large battery jar. Under the mouth was placed an agar covered Petri dish. At the end of one hour the dish was removed, covered, and allowed to remain at room temperature for several days. Abundant growth took place.

In a repetition of this experiment growth was obtained by allowing the fog to settle on plates exposed at the end of one hour and thirty minutes, but no growth was obtained after two hours, nor after four and six hours. This agrees with the results obtained by Stern,³⁶ who states that ordinary dust particles settle in still air in from one hour and a half to three hours, and can only be kept afloat by air currents of from ten to thirty millimeters per second. Very fine particles still containing bacteria can

³⁶ *Zeit. f. Hyg.*, 1889, vii, 44.

be transported laterally by a current of 0.2 mm. per second, and kept afloat by a current of from 0.3 to 0.4 mm. per second.³⁷ The fog made evident by the light beam after a period of from five to six hours is probably composed of dried salts and albumin or mucus particles, and does not contain bacteria.

Air currents which cause the movements of these very fine particles have been shown to be of much less velocity than those which occur in well ventilated rooms where the motion is from 1 to 2 mm. per second. Air at ordinary temperatures does not produce a perceptible draught until its velocity reaches 10 cm. per second. In unventilated rooms the current is less than 0.6 mm.

It is therefore possible for spray particles containing the pneumococcus to float in the air of an unventilated room for some three hours, if we assume the rate of fall as determined by the experiments to be at least 30 cm. per hour and the head of the patient to be about one meter from the floor. With air currents of very slight intensity, however, the finer particles may be carried for considerable distances. Many of these fine droplets do not contain bacteria, so that the practical danger from a patient with pneumonia is less than appears from tests under artificial conditions. The coarse particles containing many bacteria fall rapidly, and in the case of the pneumococcus, as will be seen later, many of the suspended organisms lose their vitality in the course of one or at most two hours.

EXPERIMENT V.—In order to avoid the use of mixed cultures such as would be obtained from sputum, a pleuritic fluid containing enormous numbers of pneumococci was also employed in the studies. This fluid was obtained by injecting small amounts of sputum into the right pleural cavity of large rabbits. The injection is easily made by passing a fine needle through one of the intercostal spaces on the lateral aspect of the thorax. The animals usually die in two to three days, and if the thorax is carefully opened from 10 to 50 c.c. of clear or slightly bloody fluid can be obtained. Usually both pleuræ and the pericardial sac contained fluid.³⁸

Ten cubic centimeters of the pleuritic fluid were sprayed, and at the same time sixteen liters of air were removed at such a rate that the velocity between the baffle plates was 2 mm. per second. The control plate in Chamber A was

³⁷ Flügge, *Zeit. f. Hyg.*, 1897, xxv, 193.

³⁸ This method of obtaining a fluid rich in pneumococci was suggested to me by Dr. A. B. Wadsworth. The organisms remain alive in the serum for a long time at 0° C.

removed after thirty minutes and was found to be slightly moist. Bouillon was poured on the surface and rubbed up with a platinum needle. Loops from the bouillon were then transferred to chest-serum agar. An abundant growth of pneumococci was obtained. Plates exposed in Chamber B were removed at the end of an hour and a fresh set inserted. Cover slips which had been exposed during the same time showed numerous capsulated cocci. Bouillon was poured over one plate, rubbed up with a platinum spatula, and injected into a mouse. The animal died from pneumococcus infection. Other plates from Chamber B were exposed one, two, and three and a half hours to diffuse daylight. At the end of this time mice were inoculated from the two-hour plates, and two rabbits¹⁹ were injected in the ear vein with an emulsion from two three-and-a-half-hour plates. One mouse died twenty days later, but no pneumococci could be recovered. The other lived for two months. The rabbits did not die. A third plate after three and a half hours was covered with chest-serum agar, but no growth was obtained. Another plate from Compartment B was exposed three hours to sunlight on a slightly overcast day. Plates were made after emulsifying with bouillon and three mice injected. There was no growth on plates. One mouse died twelve days later, but no pneumococci could be demonstrated. The others did not die. Another plate was dried over calcium chloride for three hours in diffuse light. Plate cultures and animal inoculations were negative.

The results of this experiment may be considered as showing that spraying a thick albuminous fluid containing pneumococci and allowing the fine spray to dry on glass is fatal to the organisms in a very short time. If drying is prevented by collecting the particles as they fall on moist chest-serum agar, growth will be obtained if the bacteria have not been in suspension over ninety minutes. A fluid of the type used corresponds pretty closely to the thin, serous sputum of certain cases of pneumonia.

EXPERIMENT VI.—Twenty-five cubic centimeters of the thick sputum used in Experiment IV were sprayed in the box during ten minutes, using an air current of about 6 mm. per second. Control plates from Compartment A were positive. Plates from Compartment B removed immediately were negative to mice, and cultures gave only staphylococci. Cover-glasses showed numerous drops varying from three to fifteen micra in diameter. The larger drops frequently contained two or three diplococci.

One hour after spraying, cover-glasses were placed in Compartment B and allowed to remain fifteen hours. These showed only a few bacteria, less than one per square centimeter. There were, however, many masses of mucus which had fallen on the slide, most of which did not contain bacteria. No free organisms were found, all were surrounded by more or less mucus.

¹⁹ It was thought, inasmuch as the pneumococci used had been adapted to rabbits, that these animals might be more susceptible to infection than mice.

Chest-serum-agar plates, exposed in Compartment B for a period beginning one hour after the spraying was finished, gave abundant growth of staphylococci, but mice injected with an emulsion showed no pneumococci. Some plates remained in Compartment B for a number of days, but no pneumococci could be demonstrated. Dry plates remained ten days, and when covered with agar gave numerous colonies of *Staphylococcus pyogenes aureus*. Mice which were injected died, but no pneumococci could be isolated. Plates from this spraying were kept in the dark and their contents injected into mice at various intervals. Some of the animals died, but no pneumococci could be obtained.

A plate dried over calcium chloride for fifteen hours in the dark gave no pneumococci, but only staphylococci.

The results of the experiment show the rapidity with which the pneumococcus dies when sprayed in fine particles and allowed to dry. The drying seems to be an important factor, for if, as is shown in Experiment V, the bacteria are caught on moist media, a growth will be obtained. As a rule also we must assume that there are other factors at work, for the viability of the organisms after spraying sputum is certainly less than that after spraying rabbit chest-serum. The action of the mucus must be considered and possibly also the osmotic relations of the organism to the sputum may affect the pneumococcus unfavorably. It is also not impossible that a considerable proportion of the pneumococci in sputum from the later stages of the disease are not viable.

EXPERIMENT VII.—Forty cubic centimeters of fresh, thin, serous sputum, from the sixth day of the disease, one loop of which was capable of killing a mouse in forty-eight hours, were sprayed for one hour with an air current of 10 mm. per second. The control plate from Chamber A killed a mouse in forty-eight hours. Pneumococci were isolated which were positive to Gram, were capsulated, and fermented inulin. The plate was dry when it was removed from the compartment.

Plates from Chamber B, removed at the end of spraying and found to be dry, were washed with bouillon and the washings injected into two mice. One died, but no pneumococci were found; the other lived a month. Washings from these plates were sown on the surface of chest-serum agar in order to avoid the inhibiting action of the anaërobic conditions which exist under a layer of agar. Staphylococci and other unidentified organisms were obtained, but no pneumococci.

Plates dried over calcium chloride were also negative as regards pneumococci. These experiments were repeated with sputa from different cases and at different times of the disease, but the results were practically the same.

It is evident that finely sprayed sputum contains no viable pneumococci after drying on glass for one hour. The positive results occasionally obtained from the Control plates in compartment A may be explained by the thick layer of sputum which is deposited and prevents complete desiccation. The results obtained by using thin, serous sputum do not vary from those obtained when thick, mucous sputum was sprayed, although differences appear when the sputa are dried in bulk.

EXPERIMENT VIII.—The technique was varied slightly so as to transfer large numbers of the organisms and thus to keep them moist. About 100 c.c. of pleuritic fluid were sprayed, with an air current of 10 mm. per second. Fifteen minutes after spraying was completed the plates in Compartment B were removed. They were still moist. A mouse injected with an emulsion of the deposit died with pneumococci in the heart's blood. A plate was exposed to sunshine for twenty minutes, during which time it dried. A mouse injected did not die. A plate of the same series was dried over calcium chloride for thirty minutes. One-half was injected into a mouse which died with pneumococcus sepsis. The plate was dried thirty minutes more; mouse died with pneumococci in the heart's blood. A plate from the same series was dried two hours over calcium chloride; a mouse injected did not die. A broth culture was made from this plate and showed pneumococci which killed a mouse (see results obtained by Ottolenghi). Another plate was dried for three hours and a mouse injected and a broth culture made. Broth was negative and the mouse remained alive. Plates dried in air for one hour were positive; for one and a half and two hours, negative.

It is possible that when only a small quantity of fluid is sprayed there are not enough virulent pneumococci left after drying to kill the experimental animal. A certain minimum dose seems necessary to kill even as susceptible an animal as a white mouse. A larger quantity of fluid was therefore sprayed in this test, and as shown by the slightly longer life of the pneumococci as compared to Experiments V, VI, and VII, the quantity exerts some influence. The conditions approach those which occur in drying sputum in bulk (see Table II) where the life of the organism is considerably prolonged.

During this test fragments of sterilized woollen and cotton cloth, tin, and wood were exposed in Compartment B. They were removed, allowed to dry in the air for thirty minutes, and scrapings from the surface tested. The organisms on the tin and wood were dead, those on the cloth were alive, but died on drying for thirty minutes longer.

EXPERIMENT IX.—A number of observers have thought that the pneumococcus in sputum is rapidly destroyed by the bactericidal action of the mucus of the sputum. Such action has been shown to take place with nasal and uterine mucus and pure mucin.⁴⁰

⁴⁰ Wurtz and Lemoyez, *Compt. rend. de la Soc. de Biol.*, 1894; Arloing, *Jour. de phys. et de path. gén.*, 1902, iv, 291 (Bibliography).

(a) This was tested by keeping a thick, mucous sputum at $0^{\circ}\text{C}.$, as recorded in Experiment I. As the pneumococcus dies on culture media or in rabbit serum in a few days unless kept at $0^{\circ}\text{C}.$, it was thought that a better differentiation could be obtained by working at the lower temperature and in the dark. When first collected the specimens killed mice in doses of a few cubic millimeters in forty-eight hours. After fifteen days at $0^{\circ}\text{C}.$, a much larger amount of sputum was required to kill a mouse of about the same size as that used during the first experiment. At the end of six weeks mice often could be killed only by doses of a cubic centimeter of pure sputum, while one specimen was no longer virulent after twenty days. Evidently a large number of the pneumococci die in two weeks when kept in moist sputum.

As it is well established that the pneumococcus remains alive for a long period when kept in serum mixtures at $0^{\circ}\text{C}.$, a combination of this fluid with sputum should retain its virulence as long as pure sputum unless some bactericidal agent is present in the sputum. Such a mixture was therefore made and kept in Petri dishes. The sputa used were the same as in Table I. The results were as follows:

TABLE IV.
TESTS WITH SPUTUM-CHEST-SERUM MIXTURES KEPT AT $0^{\circ}\text{C}.$

Day of Test.	1	5	10	15	20	30	42	60
Sputum No. I.: + serum containing pneumococci.	+	+	+	+	+	+	+	o
Sputum No. II.: + serum containing pneumococci.	+	+	+	+	+	+	o	o
Sputum No. III.: + serum containing pneumococci.	+	+	+	+	+	+	+	o
Sputum No. IV.: + serum containing pneumococci.	+	+	+	+	+	+	+	o
Sputum No. V.: + serum containing pneumococci.	+	+	+	+	+	o	o	o
Serum alone.....	+	+	+	+	+	+	+	+

The table shows that the serum-sputum mixtures do not retain their virulence for mice much longer than the original unmixed sputum as given in Table I. In two cases, however, that of sputum No. III and No. IV, virulent pneumococci were still present at the end of six weeks, while the pure sputum was non-virulent after three weeks' preservation. This difference is possibly due to the fact that but little mucus was present in the sputum. The practical importance of these findings is that the

thin, serous sputa are likely to retain their infectious qualities somewhat longer than the thick, mucous specimens, and as the thin sputa are most easily sprayed during coughing, special care should be taken to avoid contact infections.

In order to determine the action of the mucus during spraying and after drying of the spray particles, the following experiment was planned.

(b) Specimens of sputa Nos. VI and VII were mixed with an equal quantity of rabbit chest-serum rich in pneumococci. Mice injected with the mixture died promptly of pneumococcus infection. The specimens were kept on ice in the dark, and in diffuse daylight at room temperature. The results were as follows:

TABLE V.
TESTS WITH SPRAYED SPUTUM-CHEST-SERUM MIXTURE.

		Sprayed after				
		2 days.	4 days.	8 days.	15 days.	20 days.
Sputum VI.: + serum on ice in dark. Thin, serous sputum.	Plates removed from Comp. B. immediately.....	+	+	+	+	+
	Dried in air for 30 min.	+	o	o	—	—
	" " sunlight for 30 min.	o	o	o	—	—
Sputum VI.: + serum in dark at room temp.	Same conditions as above.	+	o	—	—	—
		o	o	—	—	—
		o	o	—	—	—
Sputum VII.: + serum on ice. Thick mucous spu- tum.	Same conditions as above.	+	+	o	—	—
		o	o	o	—	—
		o	o	o	—	—
Sputum VII.: + serum at room temp. (18°–22° C.).	Same conditions as above.	o	o	o	—	—
		o	o	o	—	—
		o	o	o	—	—
Sputum VI.: Without admixture on ice.	Same conditions as above.	+	+	+	o	—
		o	o	o	—	—
		o	o	o	—	—
Sputum VII.: Without admixture in dark at room temp.	Same conditions as above.	+	o	o		
		o	o	o		
		o	o	o		
Chest-serum on ice....	+	+	+	+	+
Chest-serum in light...	+	+	+	o	—

The results of the experiments show that pneumococci die off in mucous sputum more rapidly than they do in a serum mixture and that this action is probably due to the mucus present. The pure serum used in this test preserved its virulence for weeks when kept on ice, and for eight days in diffuse light.

EXPERIMENT X.—In order to determine whether the rapid death of the sprayed organisms is due to the drying which takes place while they are suspended in the air or after they are deposited on the glass plates or other dry substances used to collect them, the following variation was made in the test.

Thirty cubic centimeters of pleuritic fluid were sprayed, using an air current of 10 mm. per second. The contents of the control plates removed at the end of the spraying killed mice in two days and gave an abundant growth on serum-agar. Plates of serum-agar were inserted in Compartment B at the completion of the spraying and the air current was continued for thirty minutes. These plates were removed in thirty minutes and a second set of serum-agar plates was substituted. These were also removed in thirty minutes and a fresh set substituted.

On the first group of plates there was an abundant growth of pneumococci which killed mice—i. e., had lost none of their virulence. The second set of plates showed about twenty colonies each. These were, of course, derived from bacteria which had been in suspension for at least thirty minutes. The third set inserted at the end of an hour after the spraying had ceased and allowed to remain for three and a half hours, showed one or two colonies of pneumococci. Cover-glasses inserted at the same time showed no demonstrable pneumococci after a long search and only small masses of deposited spray. It is evident that practically all the bacteria had settled out from a height of 38 cm. in an hour's time, and that those in suspension for that time were still alive, probably owing to their being protected from complete desiccation by the inspissated serum surrounding them. In order to extend the time during which the organisms could be suspended in the air, a sputum-chest-serum mixture was sprayed into a tall aspirating jar some 45 cm. in height. As the rate of fall in still air of fine particles containing pneumococci is about 40 cm. per hour, the jar was inverted every fifteen minutes for two hours, during which time it was exposed to diffuse light. It was then fixed mouth downward over a Petri dish containing chest-serum agar and was left for six hours in the dark. Numerous colonies of *Staphylococcus pyogenes aureus* developed, but none of the *pneumococcus*.

As shown above, the organism is alive after an hour's suspension. A second test showed that only a few pneumococci survive for ninety minutes when suspended in a fine spray in diffuse light. Such a fact is of the greatest importance from a point of view of the hygiene of those in close contact with persons suffering with pneumonic infections. It demonstrates the necessity of an abundant air supply to dilute the cloud of organisms which sur-

round a patient with a severe cough. A repetition of the same test allowing the jar to stand in direct sunlight for fifteen and thirty minutes, and then removing it to a dark room to permit the organisms to settle, showed in a very striking manner the value of sunlight as a disinfectant. Only a few colonies of the pneumococcus were obtained after fifteen minutes, and none at the end of half an hour.

SUMMARY AND CONCLUSIONS.

I. In moist sputum kept in the dark at room temperatures the average life of the pneumococcus is eleven days, though considerable variations may be noted in different specimens of sputum.

In the same sputum kept at 0° C. the average life of the organism is thirty-five days.

In sputum kept at room temperature and in a strong light the pneumococcus lives less than five days.

II. In dried sputum (a) in the dark the pneumococcus lives on an average thirty-five days; (b) in diffuse light, thirty days; (c) in sunlight, less than four hours.

III. In powdered sputum even when kept in the dark the death of the pneumococcus takes place in from one to four hours. When exposed to sunlight death occurs within an hour.

IV. No important differences were noted in the life of the pneumococcus when dried on glass, tin, or wood. On cloth the life was usually slightly longer than on non-absorbing surfaces.

V. Sprayed sputum particles remain in suspension for twenty-four hours, but all masses of a size sufficient to contain bacteria settle at a rate of about 40 cm. per hour.

VI. When sputum containing pneumococci is sprayed the organisms rarely survive for more than an hour, and often die in less time. The substance upon which the particles fall makes but little difference in the life of the organism. On cloth a slight prolongation is occasionally noted, due perhaps to the slow drying.

VII. The mucus of the sputum exerts a destructive action on the pneumococcus.

VIII. Exposure of bacterial spray to sunlight while in suspension results in the destruction of the pneumococcus within half an hour.

IX. The conclusions of practical importance which can be drawn from the facts given in this paper are as follows:—

A. The life of the pneumococcus in moist sputum is of considerable duration, the average period being less than two weeks unless the material is exposed to direct sunlight. But as such sputum does not give off bacteria even when exposed to strong currents of air, it may be considered as innocuous except to persons handling clothes, bedding, etc., which have recently been contaminated. Under ordinary conditions, however, this sputum dries in the course of a few hours or days. The dried masses retain their virulence for a long time, and if deposited on the floor or on the bedding of the patient may be powdered mechanically, and sweeping, dusting, or brushing the contaminated articles will distribute pneumococci in the air. Fortunately, however, the organisms in the sputum do not remain long in suspension and die off rapidly under the action of light and desiccation. In sunlight or diffuse daylight the bacteria in such powder die within an hour, and in about four hours if kept in the dark. The danger of infection from powdered sputum may, therefore, be avoided by ample illumination and ventilation of the sick-room in order to destroy or dilute the bacteria, and by the avoidance of dry sweeping or dusting. Articles which may be contaminated and which cannot be cleaned by cloths dampened in a suitable disinfectant should be removed from the patient's vicinity.

B. When a person suffering from a pneumococcus infection coughs, sneezes, expectorates, or talks, particles of sputum or saliva are expelled from the mouth which may contain virulent pneumococci. Such particles remain suspended in the air for a number of hours if the ventilation of the room is good. They may be inhaled by persons in the vicinity of the patient, or they may be deposited upon various articles in the room. Whether suspended in the air or dried on surrounding objects, the writer's studies show that they become harmless in a very short time,

about an hour and a half being the extreme limit, while many of the pneumococci in the spray perish in a few minutes, especially if exposed to strong light.

In the light of these experiments the risk of infection from the pneumococcus is largely confined to those in direct contact with the person whose excreta contain the organism.

The writer wishes to acknowledge his obligations to Prof. T. Mitchell Prudden for many helpful suggestions made during the course of this study.

A COMPARATIVE STUDY OF PNEUMOCOCCI AND ALLIED ORGANISMS.

THE REPORT FROM THE CENTRAL LABORATORY TO THE MEDICAL COMMISSION FOR THE INVESTIGATION OF ACUTE RESPIRATORY DISEASES OF THE DEPARTMENT OF HEALTH OF THE CITY OF NEW YORK.

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GENERAL PART.

INTRODUCTORY.

The following paper contains the principal details and results of the work carried on, under the auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York, at the Bacteriological Laboratory of the College of Physicians and Surgeons, Columbia University.

In mapping out the bacteriological work of the Commission, it was deemed advisable to institute a central laboratory to which the cultures of pneumococci and allied organisms—particularly such cultures as seemed to show variations from the classic pneumococcus type—might be sent by the various workers for further study and identification. Such an arrangement, admitting of the study of the organisms under uniform cultural conditions and by one investigator or corps of investigators, would, it was hoped, not only prove a valuable biological study in itself, but possibly minimize errors of identification and thus increase the value of statistics ultimately to be based upon the findings in the series of cases investigated.

This work was undertaken by the writer, with the assistance of Dr. J. H. Borden and Dr. C. B. Knapp. The practical agglutination work was largely carried on by Dr. Borden, and the morphological and culture tests by Dr. Knapp.

2 *Comparative Study of Pneumococci and Allied Organisms*

For the information of the various workers under the Commission, and to obtain uniformity in the scheme of investigation, a comparatively full summary of the characters of pneumococci and streptococci, and the chief points to be determined in their recognition and differentiation, as well as suggestions as to the best methods of isolating them in pure culture and preserving their vitality, was sent from the central laboratory to each independent worker. The workers were also furnished with blanks ¹:

INFORMATION TO BE OBTAINED ABOUT PERSONS FROM WHOM SPECIMENS ARE TAKEN.*

Case No. Date:

Age: Sex: Race:

Residence: City Country

Occupation (in city or country):

Condition of Health:

Present State: Condition of naso-pharynx, mouth, bronchi and lungs, and middle ear?

Past History: "Cold," catarrh, laryngitis, bronchitis, or any inflammation with which pneumococcus may have been associated?

Is person subject to "colds," bronchial affections, or middle-ear trouble? ...

Does person use alcohol or tobacco?

Family History: Have members of family (mother, father, brother, or sister) suffered from an attack of pneumonia in past years?

Association with Infected Individuals:

1. Has, or has had recently, any member of immediate family a "cold," catarrh, bronchitis, pneumonia, or any inflammatory condition with which pneumococcus may be associated (middle-ear trouble, mastoiditis, conjunctivitis)?

2. Has person otherwise associated or come into contact with any one having a "cold," bronchitis, pneumonia, or middle-ear inflammation, mastoiditis, conjunctivitis?

3. Does person often or with regularity ride in crowded tram, elevated, or steam cars?

4. Does person frequent theatres or crowded shops or classrooms?

5. Has person been exposed to poisonous gases or fumes, or other deleterious aerial agents?

Subsequent History: Condition of person 15 days subsequent to examination. Has "cold," bronchitis, or pneumonia, or other pneumococcus infection developed?

Negative Cases:

Complete clinical records should always be kept of all persons from whom specimens for examination are taken, whether organisms be obtained or not, in order to determine percentage of occurrence.

(Signature)

* This blank is to be filled out, and sent to the Central Laboratory with the culture and its description.

one for recording the data concerning individuals from whom cultures were taken, and the other for recording the morphological, staining, and chief cultural characters and pathogenic action of the organisms isolated.

These blanks were to be filled out and returned with the cultures, for the information of the workers of the central laboratory.²

DESCRIPTION OF CHARACTERS CULTURE.*

Culture No. *Case No.* *Date of isolation:*.....
Source of Culture:.....
Saliva:..... *Sputum:*..... *Throat and nose swab:*.....
Technique of Isolation:
 By Animal Inoculation. Rabbit or mouse died in..... days, hrs.
 with pure or mixed infection.
 By Culture Method. Agar plate, pour or streak.....
Morphology and Staining: (Gram's stain, Capsule stain, etc.)
 In blood or exudate of original mouse or rabbit.....

 From culture media, agar, coagulated blood serum.....

 Were similar organisms determined in smears from original specimens?

Cultural Characters:
Broth:
Agar:

Loeffler's blood serum:

Gelatin: Grows at° C. (24°, 22°, 18° C.).....

Litmus milk: Acid in days. Coagulated in days.
Inulin serum-water: Coagulation does or does not take place in days.
Pathogenicity: (Minimal fatal dose of suspension of 24-hr. agar culture in
 c. c. 0.85 % salt solution).
 How many generations removed from original isolation and on what media?

 Rabbit, weight gms., dies in days, hrs., from
 c. c., subcutaneously, intravenously.
 Mouse dies in days, hrs., from c. c. subcutaneously.
Agglutination:
 Agglutinates at 1 to in immune serum. Control test with
 homologous culture shows an agglutination at 1 to (Duration
 of observation, 3, 12, 24 hrs.)
 Agglutinates in serum from whom culture was isolated at 1 to
Other observations and remarks:
 (Signature).....

* This blank is to be filled out, and sent with cultures to Central Laboratory.

² It should be noted here that the work of each independent investigator, as recorded in his report to the Commission, represents his own findings and bacteriological identifications and not those of the workers of the central lab-

Object of the Bacteriological Study Instituted by the Commission.

—As biological knowledge widens and new technical methods are discovered, confidence in identifications based on less complete studies and more primitive methods is necessarily shaken and statistics founded on these lose their value. It is only with a re-establishing of the identity of organisms in conformity with the new requirements, or by a determination of their dissimilarity, that the old statistics can again assume importance or may be definitely discarded.

This being the case, although much work had already been done by many investigators in connection with the bacterial flora of the normal mouth, and although no reasonable doubt existed as to the presence of the pneumococci in the mouths of a certain percentage of healthy individuals, still it was thought advisable to confirm, if possible, and, it might be, extend these statistics so far as practicable in the light of recent developments in our knowledge of the biology of pneumococci and streptococci, and to apply to the recognition and identification of these organisms the newer biological and technical methods. By such a study it seemed fair to suppose the true nature of the organisms from the mouths of healthy persons could be determined, and that their identity or non-identity with pneumococci derived from persons suffering from pneumonia and various pneumococcic infections could be definitely established.

Source of Cultures Examined.—The scope of the investigation included not only the examination of cultures isolated from the saliva and naso-pharynx of presumably healthy individuals, but also of organisms obtained from individuals suffering from "colds," pharyngitis, and bronchitis.

Cultures isolated from the sputum of pneumonia patients, or from their lungs at autopsy, as well as cultures from more truly

oratory. No systematic reports have been sent by the central laboratory to those sending cultures, and their reports have not therefore been influenced by the work of the central laboratory. Such a plan has the advantage of showing the percentage of discrepancy between the identifications of the same organisms by different workers, and the disadvantage, in connection with the present series of independent papers, in the interpretation of statistics based upon the findings.

internal sources, such as the circulating blood of pneumonia patients and other septicæmic cases, pleuritic exudates (empyæma), the spinal fluid in cerebro-spinal meningitis, abscesses, otitis media, or joint infections, were also investigated, so as to afford an extensive basis of comparison, and to determine the variations in morphology and biological characters that might occur among organisms of the pneumococcus type, which were found associated with pathologic lesions and definite infections.

Plan of Biological Study.—In undertaking the investigation of such an extensive series of organisms, it was realized that only the most promising lines of study could be followed in extenso at the central laboratory, and that many suggested methods of differentiation, as well as a detailed study of pathogenicity and the pathogenic effects could not be profitably undertaken. It was hoped that these studies would be carried out in detail by the other workers under the Commission, and the value of such suggested differential methods and pathogenic characters as a means of classification be thus determined.

The work of this laboratory has been chiefly confined to the determination of morphological characters and staining reactions, and of the growth characters presented by the organism on or in the usual culture media, and their fermentative activities in the presence of various carbohydrates and especially of inulin.

The immunization of animals against different strains of organisms and the careful study of the agglutinating action of the sera of such animals on the various organisms were also given much attention, and some of the most valuable results reached have been arrived at through these studies in agglutination.

SPECIAL PART.

The great number of organisms studied—in all about 260—precludes a detailed description of any but those representing the most important types or variations from these types. The most important details from the standpoint of differentiation and classification, however, are given in outline in a table at the end of the report.

MORPHOLOGY.

The morphology of the pneumococcus is in general—especially when repeated examinations are made—one of the most valuable guides to its identity.

When typical the pneumococcus is a rather large, lancet-shaped coccus occurring in pairs and surrounded by a definite and often wide capsule, which usually includes the two approximated cocci without a definite indentation opposite their line of division. The pneumococci may, however, occur singly, or in short chains, and even fairly long chains are not infrequently met with under artificial culture conditions. These may be chiefly due to the cultural conditions or be a prominent characteristic of certain strains. Apparently the capsules of organisms making up the chains are continuous; wavy indentations are usually present, however, in the capsule of chains, and at times distinct divisions are observed.

The chief variations from the typical morphology consist either in the assumption of a more distinctly spherical coccus type, or in an elongation approximating the bacillary form. Under certain conditions of artificial cultivation a distinct flattening of the organisms, particularly those making up chains, may be seen, and even the impression of the existence of a longitudinal line of division, characteristic of many streptococcus cultures, is not infrequently gained.

The capsules under certain conditions, especially in artificial media, may be absent or not demonstrable, and in certain strains capsules apparently may not be present under any conditions.

It is readily seen, therefore, that when the capsule is absent and the pneumococcus has at the same time assumed the spherical or flattened streptococcus type, identification by morphological examination is not possible. Even if the capsules are still intact, a definite identification of such streptococcus-like forms is not permissible, since encapsulated streptococci are not infrequently met with, which by cultural, fermentation, and agglutination tests are definitely separable from pneumococci.³

³ See Hiss, *Ctbl. f. Bakt.*, 1902, xxxi, 302, and *Jour. Exp. Med.*, 1905, vii, 317.

In the opinion of the writer (Hiss), no classification based solely on morphology and the presence or absence of capsules, or the finer morphological variations of capsule formation, is of value. Practically any of the described variations may dominate one and the same culture under different or even apparently the same conditions of cultivation, and all grades may occur in capsule development from its typical formation, through all variations, to its total and apparently permanent absence.

Streptococci, as has been noted, may show capsule formation. This usually occurs, so as to be demonstrable by current capsule staining methods, only under certain and not well understood conditions, and the organisms tend to lose this character much more readily under artificial conditions than do the pneumococci. While some strains retain their power of forming capsules through many generations, the majority, under ordinary cultural conditions, usually have no demonstrable capsule.

Conditions Favoring Capsule Development and Demonstration.—The most favorable conditions known for the development of the pneumococcus capsules are found in the body fluids of man and animals suffering from pneumococcus infection. For instance, capsules may be demonstrated with ease by the usual methods in the blood, serum, and inflammatory exudates of the infected rabbit and white mouse, which are among test animals the most favorable for these experiments. Capsules may be equally well-marked in the fresh sputum of pneumonia patients, especially in the early stages of the disease, and in the exudates accompanying such pneumococcus infections as meningitis, otitis media, and empyæma. In sputum and the exudates of the various localized infections, the organisms are, however, frequently degenerated or under chemical conditions unfavorable for capsule staining, and satisfactory results are not then easily to be obtained.

The same is true of the scrapings from lungs of patients dead of pneumonia, often even in the state of red hepatization.

In artificial cultivation, if the nutrient medium is not milk or does not contain serum (serum, serum-agar, Loeffler's

coagulated blood-serum), capsules, by the ordinary methods of preparing and staining, are not usually to be demonstrated.

Capsules may, however, with much regularity be demonstrated on pneumococci from agar, broth, or almost if not all artificial media, irrespective of the length of time the organisms have been under artificial cultivation, if beef or rabbit serum is used as the diluent when they are spread on the cover-glass for staining. The copper sulphate or potassium carbonate method will then stain them readily.⁴

Capsules are usually not so readily demonstrated on streptococci, no matter what their source, and with no regularity, as compared with pneumococci. They may, however, often be demonstrated by the use of the potassium carbonate method, when the organisms have been grown in sugar-serum media or on ascitic-agar and then smeared on the cover-glass with serum. In some cultures the capsules are quite as sharp and well-defined as those of pneumococci, in others they are less well-marked, and in some appear as if in a semi-solid state and on the point of dissolving. Whether the capsules noticed on certain streptococci are of the same nature as that of the pneumococcus is at present open to doubt.

GENERAL CULTURAL CHARACTERS.

The growth characters on the usual culture media, as was to be supposed from our own previous work and the publications of other investigators, have afforded no practical basis for the differentiation of pneumococci from various simulating organisms and from streptococci. Routine tests were, however, made in neutral infusion-broth and gelatin, and on neutral infusion-agar and Loeffler's coagulated blood-serum, and in litmus milk. With the exception of gelatin and Loeffler's serum, no constant characters of diagnostic and differential worth were developed.

On Loeffler's coagulated blood-serum the pneumococcus usually develops into moist, rather watery discrete colonies which

⁴ See Hiss, *ref. cit.*

tend to disappear after some days, while the streptococcus colonies, though also discrete, are usually drier and slightly whiter in appearance than those of the pneumococcus. The colonies of these two organisms, however, often so closely simulate each other that they cannot definitely be distinguished. The Loeffler serum has, however, been of value in aiding in the separation of the so-called *Streptococcus mucosus* from pneumococci. *Streptococcus mucosus* grows on this medium in a definite, smooth, watery layer, with fairly even edges, while the pneumococcus, as above noted, usually occurs in discrete watery colonies; and even when these colonies coalesce, there is not the same smoothness to the surface and edges of the growth as is shown by that of *Streptococcus mucosus*.

Gelatin has been useful in determining the limit of low-temperature growth of the cultures, and in identifying *Streptococcus mucosus* cultures.

Many cultures of pneumococci will not develop in gelatin at temperatures lower than 22° C. Others, however, even when freshly isolated, will grow fairly abundantly in gelatin at this temperature. This is true not only of typical organisms from pneumonic sputum and other pathologic sources, but equally so of organisms from the mouths of healthy persons, so that in these peculiarities of growth or non-growth at 22° C. or below, the mouth organisms have been found to agree, and in about the same proportion, with pneumococci from pathologic sources.

Streptococci and other organisms from the mouth, which are, so far as determinable at present, not pneumococci, usually grow readily and abundantly in gelatin at 22° C. or below. As their growth is generally closely similar to that of the pneumococcus in gelatin, this test is of little value in differential diagnosis. In the case, however, of *Streptococcus mucosus* the growth along the puncture in neutral infusion-gelatin, after some days' development at ordinary room temperatures, is readily to be distinguished from that of the pneumococcus and *Streptococcus pyogenes*. Instead of being a growth made up of fine discrete colonies representing the original line of inoculation, as in the case of pneumococci and of streptococci of various descriptions, the

growth of *Streptococcus mucosus*, especially in the deeper portions, where it seems to develop most abundantly and characteristically, is made up of larger globe-like colonies, which give one the impression that slight fluidification of the gelatin has taken place. This appearance is probably due to the large amount of capsular substance developed by this organism and is one of its most constant and distinctive characteristics. Cultures of this organism obtained by us from various sources, in the early days of our work, have continued to display this character and their typical growth on Loeffler's or other serum media, without the least noticeable change. This indicates that, no matter what the evolution and relationships of this organism may have been or are, the characters now distinguishing it from pneumococci are certainly in most instances of a markedly constant character, and that it is not apt rapidly to assume the characters of typical pneumococci on the one hand, or streptococci on the other. Observations indicating such a change may possibly be based on a mistake in primarily identifying a more or less confluent Loeffler's serum growth of a true pneumococcus with that of *Streptococcus mucosus*.

FERMENTATION TESTS—ACID PRODUCTION.

When we turn from the cultural characters as developed on and in the usual culture media—which are of so little aid in differentiating pneumococci from streptococci—to certain of the physiologic processes of these organisms, as indicated by their action on carbohydrates, the field of investigation is widened and decidedly more promising. In a series of investigations carried on by one of us (1) some four years ago, but only recently published in detail, it was shown that pneumococci have marked fermentative abilities, and that these are of wider scope than those possessed by the typical *Streptococcus pyogenes* and probably many other unclassified streptococci.

Pneumococci, it was shown, produced acid with ease from monosaccharids, disaccharids, and such complex saccharids as dextrin, glycogen, starch, and inulin. Streptococci, on the other hand, as represented by various supposed strains of the *pyogenes*

type, although producing acid with much regularity from some of these carbohydrates, usually did not have the ability to ferment starch and glycogen with the same regularity and ease as the pneumococci, and never, so far as the observations on about fifty cultures indicated, were able to produce acid from inulin. Inulin fermentation was therefore looked upon as a definite character of pneumococci, and probably as a valuable aid in differentiating atypical pneumococci from the non-inulin-fermenting *Streptococcus pyogenes*. Whether other species of streptococci or other lancet-shaped organisms simulating pneumococci possessed such an inulin-fermenting ability was not at that time determined.

The medium used as a basis for these fermentation tests was serum-water, composed of beef-serum one part and distilled water two or three parts, to which one per cent. of a five per cent. solution of highly purified litmus was added. The medium was then heated to 100° C. for a few moments and the various carbohydrates added in the proportion of one per cent., after which it was sterilized in the regular manner on three consecutive days.

In the present investigation, this same medium has been used as the nutrient base in the fermentation tests, which have included a study of all the organisms in dextrose, lactose, maltose, saccharose, raffinose, dextrin, glycogen, and inulin, as well as the alcohols mannit and dulcitol.

A careful testing of the organisms sent to us by the various workers, as well as those isolated by ourselves during this work, has again demonstrated the inulin-fermenting power of pneumococci, and the value of this simple test in separating them from typical pyogenic streptococci as well as from various definitely encapsulated cocci which closely simulate the true pneumococcus. On the other hand, certain inulin-fermenting organisms have come to hand which we have not so far been able satisfactorily to identify either as pneumococci or streptococci of the pyogenes type. Some of these are definitely neither pneumococci nor streptococci of well-marked type, while others have a definite pneumococcus morphology but no capsule, or are

typically streptococci, the members of the latter being in general spherical and often occurring in long chains. These organisms for the most part lack capsules or only possess poorly developed ones. It is worthy of note that most of these atypical organisms were isolated either from the circulating blood of patients, or from some other internal source, or in many cases from pneumonic or other lungs at autopsy. The question of their probable identity will be considered more in detail in connection with the results obtained by agglutination tests in immune sera.

The chief point brought out by these fermentations, so far as its bearing on the immediate problem before the Commission is concerned, is that organisms morphologically of the pneumococcic type from the mouths of healthy individuals correspond absolutely in their fermentative characters with typical pneumococci from other sources, and thus a definite link is added to the chain of their connection with true pneumococci.

In studying organisms of the same or closely similar morphology, particularly such organisms as cannot be separated from each other by morphological or cultural characters of diagnostic value, two methods of great value have been developed during late years—fermentation tests, and the test for agglutination in immune sera. By these two methods differences in organisms have been discerned that were hardly suspected, or only guessed at by earlier investigators. In using such methods, however, which deal with the truly physiologic activities of organisms, care and patience must be expended upon prolonged observations and repeated tests. Due regard must be paid to the fact that transient and, it may be, permanent modifications may occur in the physiologic functions of organisms by exposure to unusual or adverse environment, and observations, therefore, based on short or superficial studies or tests of such organisms may be misleading. These changes are usually to be noted in the suppression or weakening of functions, upon which fermentations, agglutinations, and pathogenicity depend, and hardly extend to the acquisition of totally new functions, which probably are the product of a much more gradual and prolonged process of evolution and adaptation. These facts are well-

known, but attention is called to them here in connection with the interpretation of fermentation and agglutination results which, unless considered from this broad standpoint, are often confusing or lead to false conclusions. As an example, we may use the variations in the rate of fermentation of inulin by various cultures of pneumococci. A culture in full possession of this faculty may produce marked acid within from eighteen to twenty hours, while another culture, if tested only over five or six days, might be thought not to ferment inulin; after a longer time, or in repeated tests, however, this function will, so far as our experience goes, eventually reveal itself. Variations in the rate of fermentation are often marked in the same culture, differences of days in the time required for complete fermentation often being noted.

In the absence of all information as to the reaction of organisms in specific immune sera, fermentations seem, therefore, the safest guide, other things being equal, to the identification of organisms. Where fermentations have been carefully studied and the identity of organisms thus determined, one can usually predict with much certainty what the results of tests in immune sera will be. However, it must be remembered that variations in agglutinative functions also occur and may be fairly permanent, thus leading to false conclusions, unless, on the other hand, due regard be paid to the other biological characters as means of identification, and to the source of the culture. Changes in the limit of agglutination of undoubtedly the same species or even the same culture, after exposure to certain environments, are well known to those familiar with typhoid agglutinations, and in considering, in the following section, some of our agglutination results, the same phenomenon will be noticed to occur among the pneumococci.

AGGLUTINATION TESTS.⁵

In undertaking the systematic comparative study of the agglutinations of pneumococci and allied organisms from various

⁵ The table has been placed at the end of the paper.

sources, the workers under the Commission had to enter a practically uncultivated field of research. No guiding statistics of value existed and the technical procedures, at least those available for such an extensive study, had not been worked out. The same statement is equally true of streptococcus agglutinations and agglutination technique. Agglutination tests with both pneumococci and streptococci, where the usual broth cultures (either with or without sugar added) or emulsions from agar had been employed, had in the experience of most workers been found not only technically unsatisfactory, but had given varying and often contradictory results.

After a few trials of the older methods it was obvious, therefore, if advances were to be made in our knowledge of the agglutinations of these organisms, that a new as well as a simple and easily handled technique had, if possible, to be devised.

The first problem was to get the organisms in a proper and dense enough emulsion for observation of agglutination, and the second to obtain them in the biological state in which they would respond definitely and with regularity to the action of the agglutinins, for with the old procedures, as stated, only irregular and unsatisfactory agglutinations had been obtained. The only technique already in use which in any degree conformed to these conditions was that recommended by Wadsworth.⁶ This is valuable and reliable, but when a long series of organisms, as in this comparative study, have to be tested against the same or various sera, almost a prohibitive amount of time and energy must be consumed, when following this technique, in centrifugating and preparing the organisms for the tests.

A method which met the requirements was finally worked out by us. For purposes of reference it is given here in the words of the original paper which has but recently been published (2):

"The method gives results and appearances which are entirely comparable to those familiar in typhoid, dysentery, and various other agglutinations, and is simple and particularly available for the study of pneumococci and streptococci and other organisms which ferment carbohydrates, but which thrive poorly or die out rapidly in the usual media. The streptococci often, as is well

⁶ Wadsworth, *Journ. Med. Research*, 1903, x, 228.

known, grow in broth, either with or without sugar, in flocculi or thickly matted masses entirely useless for agglutination purposes. By the proposed method usually a fair and often a good and satisfactory emulsion may be obtained, from which agglutinative limits may easily be determined.

"The medium used should be a one- or two-per-cent. peptone broth made from meat infusion, which has been brought to neutrality before boiling and coagulation. After filtration for clearing, one per cent. of dextrose (or other sugar fermentable by the given organism) and one per cent. of calcium carbonate are added. If the medium be acid, the latter salt will of course bring it to neutral. The calcium carbonate may then be well distributed throughout the broth by shaking and the emulsion rapidly decanted into tubes or preferably small Erlenmeyer or Florence flasks, say 100 cubic centimeters to 150 cubic centimeters in each. These are sterilized on three consecutive days at 100° C. in the usual manner. The flasks after inoculation are placed at 37° C. and are thoroughly shaken once or twice a day to neutralize the acid formed and to break up the chains and masses in the case of streptococci. The growth may be sufficient for purposes of agglutination in two days or even in one day, but as a routine up to the present time we have employed a three or four days' growth, which seems to give more uniform results and more marked agglutination.'

"About an hour before using for agglutination tests the culture should be thoroughly shaken and the calcium carbonate and larger clumps, if present, allowed to settle during this time. The sample to be tested should then be taken from the upper portion of the fluid; or the cultures, after shaking, may be centrifugated for a few minutes. This centrifugalization, however, is not necessary if one remembers that a slight primary deposit may occur which is not due to agglutination. . . .

"Routine agglutination tests are made by adding one cubic centimeter of the serum dilution to one cubic centimeter of the emulsion in small test-tubes. The tubes are placed at 37° C. for two or three hours—after which time the agglutinations are often practically complete—and then transferred to the ice-box to prevent growth taking place and permit of the further deposition of the clumps of agglutinated organisms, and the final control reading made after eighteen to twenty-four hours. . . .

"Spontaneously agglutinating cultures, of course, are met with among the pneumococci, and are frequent with streptococci, but even with these the limits of agglutination can be determined with much certainty if careful comparison with the control is made."

In the course of our work many hundreds of serial tests have been made by this method, and it has proved of great value not only in the ease of application but in the comparative uniformity of results given by it. Readings up to eight hundred and over

' Such cultures may then be preserved in the ice-box. We have tested them frequently in the same sera from day to day, and have found little or no change in their limit of agglutination even after weeks.

are not rare in the sera of rabbits immunized to pneumococci, and the serum of streptococcus immune rabbits has in some instances agglutinated the homologous streptococcus cultures in dilutions over 6400. Reported agglutinations of pneumococci made by the usual method rarely indicate an agglutinating power of the sera over 100, and such readings are obtained with no uniformity. Our success in getting such high agglutinations has probably not only been due to our method of making the test, but in part, at least, to the use for inoculation of the animals of mass cultures obtained by growing the organism for this purpose in the calcium-carbonate-glucose broth. Our animals, although usually very gradually immunized at first, eventually received large doses of these growths intravenously, and for the most part survived them and remained in good condition. Rabbits, however, immunized against *Streptococcus mucosus* in several instances became much emaciated and eventually died.

A series of animals was immunized with pneumococci from pneumonic sputum, and from the mouths of healthy individuals, also with *Streptococcus pyogenes*, *Streptococcus mucosus*, and various other organisms of peculiar types. The sera obtained from these animals and from normal rabbits afforded the basis for an extended study of the agglutination reactions of the various cultures.

All the organisms of our series have been tested against the immune pneumococcus sera, and their agglutinations controlled by tests in normal rabbit serum and normal salt solution, and a careful comparison made with the results given by the homologous immunizing cultures.⁸

Pneumococci as a rule, no matter what their source, do not agglutinate in high dilutions of normal rabbit serum, rarely over 1:10 to 1:20, nor have they been found to agglutinate to any

* There may be a slight false primary clumping in all tubes of the series, including the controls. These clumps are apt to disappear later. Even if they persist they do not settle out rapidly, and the specific agglutination is readily distinguished from them. Such occurrences, however, should make one exceedingly cautious in accepting results reported in pneumococcus and streptococcus agglutinations when the hanging-drop, microscopic-test method alone has been employed.

marked extent in streptococcus immune serum or, with the exception of *Streptococcus mucosus* serum, in the immune serum obtained by inoculation with other organisms of various types.

Nearly without exception, however, organisms previously recognized by morphological, staining, and fermentation tests as distinctly of the pneumococcus type, have been found to agglutinate in pneumococcus immune serum, and the results obtained, taking the series of these organisms as a whole, have shown remarkable uniformity in degree of agglutination and a close approximation to that given by the homologous organism. This is true not only of pneumococci from recognized pathologic sources, such as pneumonic sputum, etc., but of the organisms of pneumococcic type from the mouths of supposedly healthy persons. The same results are obtained in the sera of animals immunized against these latter as in the sera of those immunized against pneumococci from pathologic sources. In other words, the agglutination tests have fully confirmed the complete identity, which was presaged by the fermentation tests, of organisms of pneumococcic type, from the mouths of healthy individuals, with those from pathologic sources. Further than this, these tests have also reinforced the evidence given by fermentation tests, namely, that there are distinctly encapsulated, Gram-positive organisms which may be met with and which simulate pneumococci too closely for morphological separation, but which are, nevertheless, according to agglutination and fermentation tests, separate and distinct from pneumococci. These remarks do not apply to *Streptococcus mucosus*, which is peculiar in its agglutination reactions, showing only moderate agglutination in the sera of animals, which according to all standards are highly immunized against it. Agglutinations of 1:20 to 1:50 generally abruptly mark the limit. In pneumococcus immune serum it shows little or no agglutination. On the other hand, pneumococci agglutinate in *mucosus* immune serum in very high dilutions. From this fact and the fact that the fermentations caused by these two organisms are, so far as we know, coextensive, we have been practically forced to the conclusion that *Streptococcus mucosus* is not a distinct species, but a variety of

pneumococcus, which is, however, very firmly established in the possession of certain morphological peculiarities, especially as relates to the abundant production of mucinous or capsular material. Its peculiarities in agglutination, or rather non-agglutination, may be, we have thought, closely connected with the over-production of this special mucous or mucinous material and its solution in the culture fluids—in other words, that this material may have a combining or inhibitive action on the agglutinins. In cultures centrifugated and washed in salt solution we have, however, failed to increase markedly its agglutination, though some slight increase did take place. What the explanation is we at present do not know, but the fact that pneumococci agglutinate to such a marked degree in *Streptococcus mucosus* immune serum argues a close relationship between the two.

IMMUNE SERA.

	Pneum. "1."	Pneum. "3."	Pneum. "23."	S. muc. "7."	S. muc. "7-a."	S. pyog. "1."
Pn. "1".....	400-800	200-800	400-800	200-800	400	0-100
" "3".....		400-800			200-800	0-100
" "23".....		100-800	200-800	100-200		
" "45".....		400-800	100-200	200-800	100-400	
" "E. 1"....		100-800			100-200	
" "E. 32"...		200-800		100+		
" "E. 55"...	100-400		400-800	200-400		
" "N. 7"...	200-800		800	200-800	200-800	
" "N. 17"...	200-800		800	200-800	200-800	
S. pyog. "1".....		200-800				800-6400
S. muc. "7".....		0-10		10-100	10-200	0-50
" "22".....		0-10		10-50	0-50	—
" "30".....		0-20		10-20	0-20	0-20
" "55. a".....	0-10			10-50		
" "Br.".....	0-10			10-50		

The immunizing organisms Pn. "1" and Pn. "3" are from pneumonic sputum: Pn. "23" is from the normal mouth; and the other cultures from normal mouths and various pathologic sources.

The italics indicate the agglutination in the homologous serum.

When two numbers are used to record the results of a test, the first indicates the last dilution in which full precipitation of the agglutinated organisms occurred, and the second the highest reading with a hand lens.

The results of control tests in normal rabbit serum are not recorded in the table. A few of the pneumococcus cultures showed a very slight agglutination at 1:10. The *Streptococcus pyogenes* culture agglutinated at 1:100. None of the *Streptococcus mucosus* cultures showed agglutinations at 1:10.

The above table illustrates the agglutination of pneumococci

in pneumococcus and *Streptococcus mucosus* immune sera, as well as in *Streptococcus pyogenes* immune serum and normal rabbit serum. The agglutinations of *Streptococcus mucosus* and *Streptococcus pyogenes* are included in the table for comparison.

Before leaving the subject of the agglutination of pneumococci, which are typical morphologically as well as in their fermentation reaction, it must be noted that certain of them when compared with the homologous organisms in immune serum show a very low grade of agglutination. A glance at the full table at the end of the report will show that such organisms are usually not from the normal mouth or from pneumonic sputum, but from some more internal source, such as the blood, or some chronic and deep-seated lesion, or lungs at autopsy. These results are in line with those found in the case of typhoid bacilli from the circulation and from more chronic lesions, as well as those grown artificially in immune serum, and are probably an illustration of the modification of function by environment.

Of the organisms examined by us which do not ferment inulin and which are of typical streptococcus morphology, or even of diplococcus type and slightly lancet-shaped but non-capsulated, little need be said here. The results of their agglutination tests will be found in the appended table. It is simply worthy of note that some streptococci show marked agglutination in normal rabbit serum and naturally, therefore, also in anti-pneumococcic serum, and that unless controls be made false conclusions might be drawn from this. In their homologous sera they may agglutinate in high dilutions. Another point made clear is that various streptococci of the same morphological and possibly even the same fermentative and cultural characters do not, with anything like the uniformity displayed by pneumococcus cultures, agglutinate in a given streptococcus serum. This indicates the possibility of a future satisfactory classification on this basis.

The other organisms of the series, which have not as yet been mentioned but which deserve especial attention, are inulin-fermenters of pneumococcus morphology but without capsules, other lancet organisms not so definitely of the pneumococcus

type, and organisms apparently of definite streptococcus morphology, all of which ferment inulin.

Some of the first variety, if not all, are undoubtedly pneumococci, and agglutinate in pneumococcus immune serum, though usually not in high dilutions, and there seems no reason for placing them in a separate class.

Other inulin fermenters, which are small organisms, at times showing lancet morphology, but which are usually non-capsulated, and vary in some of the less important features in artificial media, it is difficult to classify, as they show little or no agglutination in pneumococcus serum. Whether they are modified pneumococci or should be placed in a distinct class, our studies at the present writing have failed to determine. Their agglutinations are recorded in the table. Apparently all of them are not of the same type. If the requisite time had been at our disposal, a special study of the pathogenic effects of these organisms on the one hand, and on the other of the modifying influence on the organisms themselves of a residence in the animal body, would have been undertaken.

Our attention has, however, been especially directed to certain non-capsulated, inulin-fermenting cultures kindly sent to us by Dr. Charles Norris and Dr. Leo Buerger. Two of the cultures from Dr. Norris show variations from the classic pneumococcus type. One of these, known by us as "Nor. 199," only varies from this type in not possessing a capsule. Its morphology otherwise is typical, and its growth in rabbit blood-agar cultivations corresponds absolutely to the growth given by the vast majority of pneumococci in this medium. It agglutinates to some extent in pneumococcus immune serum and is probably a true pneumococcus.

The other culture, "Nor. 102," has no capsules and shows a less typical lancet morphology. Its growth in blood-agar plates is not that of the most diagnostic type, but corresponds to a type seen, nevertheless, among certain otherwise perfectly typical pneumococci. No agglutination in pneumococcus serum has been shown. The weight of evidence is, however, in favor of its being a temporarily or permanently modified pneumococcus.

Dr. Buerger's culture, known as "Streptococcus No. 7," ferments inulin. Its morphology is in general of streptococcus type, but lancet forms at times may predominate. Very narrow capsules have been observed by me on some cultivations of this organism. This organism does not agglutinate to an appreciable degree, according to our macroscopic tube agglutinations, in anti-pneumococcus sera, but gives high agglutination in its homologous serum. Some cultures of pneumococci agglutinate in this serum even in high dilutions. In blood-agar plates the colonies appear brown or dark-green surrounded by an opaque area—the whole plate assuming a distinct greenish tinge, which is more marked, if anything, than the tinge occurring in most pneumococcus plates.

The organism is definitely not a streptococcus of the pyogenes type, and probably not a true streptococcus, unless it is of the type described by Schottmüller as *Streptococcus mitior viridans*. Not having had the opportunity of examining Schottmüller's original cultures, it is, of course, not known to us whether they ferment inulin or are non-inulin fermenters of streptococcus type, such as have been met with in our present studies, and which also produce a greenish color but no clear lysis in blood-agar plates. The true status of this Buerger streptococcus (?) we have, therefore, up to the present been unable satisfactorily to determine, though it may be an atypical pneumococcus.

GROWTH CHARACTERS IN BLOOD-AGAR.

Schottmüller (3) in 1903 and, independently of him, Rosenow (4) in 1904 called attention to certain reactions caused by pneumococci and streptococci when growing in agar with which human or animal blood had been mixed.

Schottmüller stated that pneumococcus colonies developing in this medium usually became of a greenish tinge, and were surrounded by a zone of opacity of a greenish color. Streptococci of the erysipelatos type did not assume a dark or greenish tinge, and were surrounded by a distinct clear zone due to complete lysis of the red corpuscles and change of the hæmoglobin. Rosenow's observations confirmed these.

Schottmüller further described a form of streptococcus which he designated *Streptococcus mitior viridans*. This was less virulent than the other type and was said to be usually associated with rather chronic lesions and septicæmias. It was described as of streptococcus morphology and non-capsulated. In blood-agar plates it simulated the pneumococcus, but the greenish tinge was less intense. Little or no hæmolysis occurred. What the true nature of these organisms is, is in doubt, as nothing is known of their fermentations and agglutinations.

In our series of organisms we have found the action of pneumococci, as a rule, to conform to Schottmüller's description, but usually with a deep-brown or reddish tinge to the colonies rather than a definite green, the opaque area of partial hæmolysis (and precipitation (?)), but not clearing, and of yellowish or greenish tinge being present.

Some organisms of our series of true non-inulin-fermenting streptococcus type conformed to his description of *Streptococcus erysipelatos* with the clear zone surrounding their colonies and no greenish tinge. Other non-inulin-fermenting organisms of general streptococcus type, some capsulated (see P. and S., No. 8) and some not capsulated, gave the typical pneumococcus or *Streptococcus mitior* pictures, which are really not to be distinguished from each other.

Our unclassified inulin-fermenting organisms either produced no lysis or change in color of the medium, or gave rise to appearances closely suggesting or absolutely corresponding to those given by pneumococci.

Whether this can be depended upon as indicating their pneumococcus nature, one would hesitate to say in face of the fact that so many non-inulin-fermenting, non-agglutinating, and definitely not pneumococcus cultures also give rise to these appearances.

In streak cultures on slanted rabbit-blood-agar in tubes,⁹ the writer, in making a comparative observation on about two hun-

⁹ This medium is made by bleeding a rabbit from the carotid artery, through a sterile canula and rubber tube, directly into tubes of fluid sterile agar (kept at from 45° to 50° C.). About one-half to one cubic centimeter of blood should be added to each tube. The blood must be mixed immediately with the agar (to prevent clotting), and the tubes slanted and allowed to harden.

dred pneumococci, streptococci, and miscellaneous unidentified organisms, noted certain appearances which, when prominent, seemed to be practically diagnostic of the pneumococcus cultures.

Streak cultures of pneumococci on mixed blood-agar grow at first with the usual characters seen on serum media. The growth, however, appears usually of a rather dirty yellowish tinge, and the blood-agar in immediate contact with the growth and at times for some distance from it takes on a yellowish rather opaque look, due to decolorization and probably to a slight precipitation from acid formation. The variations met with are usually in the direction of a less typical growth layer, combined with more decolorization and hæmolysis, though the medium remains opaque.

During the first few days of growth neither of these appearances is characteristic. When, however, the tubes have remained in the incubator some days, the majority of the pneumococcus cultures take on a distinctly characteristic appearance. Most of the growth disappears, leaving the general decolorized dirty-yellowish area, while definite raised colony-like nodular masses remain, which usually are of a brownish-black or dark-red color, as if the masses had become distinctly stained with the blood pigments. One gets the impression of a blistered painted surface. None of the streptococci or other organisms, so far as determined by this test, assumed this appearance. Whenever this appearance was noted, the organisms were found to be true pneumococci. The cultures of pneumococci, giving less typical growth and more extensive decolorization of the medium, retained the more even appearance of surface and did not become nodular. So far as we know, these cultures are true pneumococci, but, it may possibly be that they are modified *Streptococcus mucosus* cultures which had not previously been recognized. The *Streptococcus mucosus* growth, though at first prominent and moist and quite characteristic on this medium, soon practically disappears. Traces of decolorization may or may not be prominent. One culture, however, presented an appearance similar to that of the pneumococcus.

PATHOGENICITY.

This phase of study has not, as was stated earlier, been taken up with any regularity in our comparative study. Simple tests of the pathogenicity of organisms, especially such organisms as pneumococci and streptococci, usually give little information of value in classification, and even definite studies of lesions caused by such organisms are not often of diagnostic aid.

It has been generally demonstrated by all investigators that pneumococci as a class, especially when freshly isolated, are usually pathogenic, although in varying degrees, for white mice and rabbits; while streptococci, even from severe lesions in man, may show little or no pathogenicity for mice and rabbits and other test animals. Both of these organisms, even when primarily virulent in high degree, tend to lose this character when cultivated on the usual artificial media. Any tests, therefore, to determine the pathogenicity of the organisms, when they arrived at our laboratory, would have led to little or no information of value as to the original pathogenicity of the cultures. This work was left in the hands of the investigators who made the isolations.

All that need be said here is that so far as our own cultures are concerned and so far as we can gather from the information sent to us by the workers in other places, little or no difference has been determined in the relative pathogenicity of organisms of pneumococcic type from the mouths of healthy individuals and those from persons suffering from pneumococcic infections.

If such pathogenicity for animals indicates in any way the grade of pathogenicity of organisms for man,—a supposition always open to grave doubt,—and if, especially, these organisms be found to linger in normal mouths during the summer months, the possession of virulency by organisms from healthy individuals is a matter of interest and importance in the consideration of the mouth as a nidus of these infective agents and the possible and probable ways of their preservation and dissemination.

REMARKS ON THE CLASSIFICATION OF THE ORGANISMS INVESTIGATED.

An attempt, at this stage of our work, to classify definitely all the organisms which have been received by us would be ill-advised. Many of these have but recently come into our hands, and when not responding definitely to the tests and showing easily recognized and diagnostic characters of some one of the well-known types, our acquaintance with them has been too limited to warrant an attempt at classification. Any points, however, so far determined about their general morphology and biology, are recorded in the table.

The following tentative classification is given for purposes of reference and as an illustration of some of the types met with:

A. *Inulin fermenters.*

1. Typical pneumococcus morphology. Typical capsules. Agglutinate in pneumococcus immune serum. Growth on or in blood-agar usually typical. *Typical pneumococci.*
2. Typical pneumococcus morphology. No capsules determined, even in body fluids of infected animals. Agglutinate in pneumococcus immune serum but usually not in very high dilutions. Growth in blood-agar same as that of typical pneumococci. *Pneumococci.*
3. Atypical morphology. Lancet-shaped organisms occur, but morphology approximates streptococcus type, or some cultures may be composed of small lancet-shaped diplococci. Capsules, usually not well-marked, may be present. Growth in blood-agar plates may or may not be typical. Do not show nodular growth on blood-agar streak cultivations. Organisms mostly from pneumonic lungs at autopsy or from some internal body source. Do not ordinarily show diagnostic agglutinations in pneumococcus immune serum, but may agglutinate in homologous sera to fair degree. Pneumococci do not, as a rule, agglutinate diagnostically in these sera. Probably a mixed group, some of which are presumably true but atypical pneumococci, or atypical *Streptococcus mucosus*.
4. Morphology practically like that of the pneumococcus,

but chains are more frequent and the elements are more usually spherical. Capsules typical and well-marked. Agglutinates only in low dilutions of homologous immune serum or in pneumococcus immune serum. Pneumococci agglutinate in high dilutions of the serum of animals inoculated with this type. Separated from pneumococci by peculiarities of growth in gelatin and on Loeffler's and other serum media. *Typical Streptococcus mucosus*; probably a variety of pneumococcus.

B. Non-inulin fermenters.

5. Capsulated organisms simulating pneumococci, but of general streptococcus morphology. Capsules fairly constant on artificial media. Ferment many sugars, but not inulin. Simulate pneumococcus growth and reactions in blood-agar plates, but do not show nodular growth on blood-agar streak cultivations. Do not agglutinate in pneumococcus immune serum. Agglutinate in moderate dilutions of homologous serum, in which serum the pneumococcus does not agglutinate. *Streptococcus mitior viridans* (?).
6. True streptococcus morphology. Ordinarily capsules are not demonstrable. Ferment many sugars but never inulin. Colonies in blood-agar plates not green-tinged, but surrounded usually by a well-marked clear zone. Apt to agglutinate in normal rabbit serum, even up to 1:200. Agglutinate markedly in homologous sera. Pneumococci are practically not agglutinated by these sera. *Typical Streptococcus pyogenes (erysipelatos)*.
7. Streptococcus morphology. May or may not have capsule. Ferment many sugars. Orange growth along puncture in Welch's and other media. Agglutinations in general not determined, but do not agglutinate in pneumococcus serum. May be mixed group.
8. Streptococcus morphology. May or may not have capsules. Sugars not fermented, with possible exception of some monosaccharids. Do not agglutinate in pneumococcus immune serum.

It should be noted in connection with this classification of the streptococci, that it is merely in outline, and that other classifications, possibly of value, are to be arrived at from a detailed grouping by fermentation reactions. Attention was called to this subject by the writer in a previous paper. A close study of agglutination, taken in connection with the groupings suggested by fermentation reactions, will probably lead to a separation of streptococci into distinct and easily recognizable groups.

So far as the whole classification is concerned, it should again be recalled that variations of a fairly permanent or even of a transient nature in the morphology and physiology of organisms may definitely interfere with their recognition, and when classifications have to be based on such delicate biological processes as are involved in fermentations and agglutinations, only long-extended and painstaking observations will lead to an eventual recognition of their true identity.

In connection with this statement and the following remarks upon statistics, it is of value to note the percentage of discrepancies between the results obtained by us from our examination of the cultures sent to the central laboratory and the results which were obtained by the various workers from their examination of these same cultures and reported to us. Out of 146 instances in which the cultures were reported as fermenting inulin, we have only been able to determine the occurrence of such fermentation in the case of 119 of the cultures, a difference of 18½ per cent. The results of our inulin determinations were, nearly without exception, supported by the agglutination tests—in only two instances, in fact, where inulin fermentation was reported and our tests gave negative results did the agglutination results seem to indicate the pneumococcus nature of the organisms. In both of these cultures the organisms had a distinct streptococcus morphology, and as streptococci frequently agglutinate in high dilutions of normal and heterologous sera these results are not determinative.

In only one instance did we find inulin fermentation when none was reported, and in this culture the morphology was perfectly typical, and typical capsules were present. Our

results show in this instance, however, that no agglutination took place in pneumococcus immune serum.

This discrepancy of nearly twenty per cent., indicating as it does the differences in identifications of the same organisms when studied by different investigators, has an extremely important bearing upon the percentage of error which in the ordinary course of events must be allowed in the consideration of statistics from various sources.

STATISTICAL.

Bacteria of Pneumococcus Type in Normal Mouths.—Enough has been said in the previous part of this paper to make it clear that there occur in the mouths of healthy persons organisms that show, according to the most refined morphological and biological tests, all the characters displayed by true pneumococci from pathological sources. No reasonable doubt can now exist as to their true pneumococcus character.

One of the fundamental objects of the bacteriological work instituted by the Commission—the solution of the problem of the nature of these organisms—may therefore be looked upon as accomplished.

Allied to this a point of great importance has also, we think, been conclusively demonstrated, namely, other organisms are to be met with which too closely simulate pneumococci morphologically to be separated from them by the most careful morphological examination or by the usual routine cultural tests, only fermentation and agglutination tests definitely establishing their nature. The futility of attempting to base statistics on purely morphological findings and staining reactions, or even superficial culture tests, must, therefore, be obvious. At the best they are but indicative.¹⁰

Another object of the Commission, necessarily secondary to the establishing of the nature of mouth organisms, was the de-

¹⁰ Old statistical statements as to the frequency of occurrence of pneumococci in normal mouths, although this frequency may be found to approximate closely present findings, must, nevertheless, in the light of these facts, lose any just claim to scientific accuracy.

termination of the frequency of occurrence of pneumococci in the mouths of healthy individuals.

For such studies to be of definite statistical value it was obvious that series of individuals living under different environmental conditions should be examined. These were indicated as follows:

1. *Normal persons*, giving history of no known contact with a source of infection and no recent history of "cold," bronchitis, or pneumonia, middle ear, conjunctival, or other possible pneumococcic infection.

2. *Normal persons*, intimately associated with patients suffering from pneumococcic infections—i.e., pneumonia, bronchitis, etc. (nurses, doctors, hospital attendants, and non-pneumonic patients in wards with pneumonia patients).

These investigations were not to be confined to one locality, but to be carried on in other places besides New York—in particular in Philadelphia, Boston, and Chicago,—thus assuming a much more general significance, and in themselves affording a basis of comparison of frequency of occurrence in different localities. The results of this investigation by the various workers will doubtless eventually be correlated and tabulated; at present they are scattered throughout the independent reports.

Before considering the results of our own investigations along these lines, attention should be called to certain factors that may influence deductions made from such findings. Considered from the standpoint of a simple illustration of the ease of general dissemination of organisms thrown off from the bodies of diseased persons by way of the mouth and nose, the finding of pneumococci in the mouths of normal individuals may seem to have great significance. Another interpretation is, however, perfectly legitimate from such simple data; in other words, these organisms may be common and permanent inhabitants of mouths, as colon bacilli are supposed to be of the digestive tract. If such a view were substantiated, the application of protective hygienic measures might assume less importance.

There seem to be two definite ways of solving this problem.

Of these the only one open to the investigator in cities is the careful examination over extended periods of the bacterial flora of the mouth of one and the same individual. In this way the permanent flora of such a mouth may soon be determined and the appearance or disappearance of organisms noted with accuracy. Even varieties of organisms of the same species may be recognized, when thoroughly investigated, by some minor biological or possibly morphological peculiarity, and thus definitely distinguished from a new invader of the same species. If, then, upon the advent of such an invader pathologic changes occurred, its association with these would be fairly well established, and the entire period of its residence could be determined. The other method of solving this problem is the investigation of individuals living in localities where pneumonia does not ordinarily occur, or of individuals not coming frequently into contact with large masses of population. As a possible means of arriving at a solution of this problem, the writer has suggested the examination of the mouths of such persons as sailors arriving from long voyages by sea.¹¹

By such methods as these the permanency of the occupation of the normal mouth by such organisms could be determined, or their definitely transient nature be shown. This will be referred to again in considering the statistics of our own investigations.

Our own investigations on the bacterial flora of the mouths of normal persons was limited to a study of the saliva from twenty-two persons not known to be suffering from any form of pneumococcic infection. In the case of fifteen of these individuals only one examination was made, and this in each instance by mouse inoculation. If the mouse did not die within a reasonable time the bacteria of the local lesions were investigated. Organisms of definite pneumococcus type were isolated from seven of the fifteen persons, i.e., in 46.6 %. The saliva from the remaining seven persons was tested repeatedly, and pneumococci were demonstrated in the saliva of six out of the seven, i.e., in 85.7 %.

¹¹ This work is now being carried on at the New York Quarantine Station, and, it is hoped, may throw some light upon the subject of the persistence of pneumococci in normal mouths.

These higher figures may be interpreted in two ways, either as representing a correction of errors due to the single application of any given method of isolating organisms, or to this in part and in part to the detection of new invaders, since the examinations were made at intervals, over several months. No matter what the actual interpretation, the prominent fact brought out is that practically every individual, at least during the winter season when exposed to environmental conditions such as those existing in New York City, acts as host, at some time or other, and probably at repeated intervals, for organisms of the most characteristic pneumococcus type. In answer to the question above proposed as to the continuous residence of such organisms as parasites of the mouths of normal persons, it may be said that it is the writer's opinion that in the majority of cases the residence of any given strain of pneumococci under such conditions is apt to be temporary rather than permanent or long continued.

Some of the reasons for such an opinion may be given: in the first place, repeated examinations carried on by all methods at short intervals may fail for a longer or shorter time to demonstrate the presence of pneumococci in the mouths of a certain percentage of normal individuals. This, taken in connection with their practically universal occurrence, indicates that organisms of this type tend to disappear from such mouths; and furthermore, even when pneumococci are found repeatedly and constantly throughout long periods, unless the strain present has some minor peculiarity by which it may be recognized from new invaders, we cannot argue that we are not dealing with newly acquired organisms at each examination.

In the second place, unless such sensitive organisms rapidly become adapted to some more or less protected nidus in the mouth or naso-pharynx, such as the tonsillar crypts or, as it seems not unlikely to the writer, to a parasitic residence in the salivary ducts, the ordinary conditions in the mouth during the taking of foods, especially of an acid nature, and the rapid osmotic changes to which the organisms in general must be subjected when food or drink is in the mouth, seem highly unfavorable for organisms so sensitive to their environment as

pneumococci undoubtedly are under observed cultural conditions. Further than this, flora already adapted may interfere with the occupation by these new forms. It seems not unlikely, therefore, that such invaders usually, unless particularly favorable conditions (temporary or permanent) for their adaptation to this new environment exist, would tend to die off after a comparatively short residence.

PNEUMOCOCCI IN THEIR RELATION TO "COLDS."

When the repeated investigation of the flora of the same mouth was taken up, we had in view not only the determination of the normal and transient bacterial inhabitants, but also of the relation of these latter to disease processes which might manifest themselves during the period of such examinations. In most instances, so far as the histories showed, no "cold," pharyngitis, bronchitis, or pneumonia, either preceded, within a recent period, the determination of pneumococci in the mouth, or developed subsequent to the invasion of the mouth or nasopharynx by these organisms. In two instances, however, the appearance and residence of pneumococci in the mouths of persons hitherto free from them were incident with the development and course of "colds." Subsequent examinations in one case showed the early disappearance of the organisms; in the other, in which the inflammatory process had been much more severe and a purulent post-nasal discharge was developed, the organism (presumably the same) had remained in the mouth up to the last examination, about a month subsequent to the cessation of all symptoms of infection. In the purulent discharge pneumococci were present in practically pure culture and in the greatest profusion.

In each of these cases, it is interesting to note as an illustration of the value of careful study in recognizing organisms, that the mouth seemed to be the permanent habitat of organisms of general pneumococcus morphology—in one instance of a Gram-positive, capsulated, non-inulin-fermenting streptococcus morphologically not definitely to be distinguished from the pneumococcus; in the other, of a typical *Streptococcus mucosus*. In both cases

these organisms were found upon the first examination, and persisted throughout the entire period of examination extending over months. The capsulated streptococcus rarely killed mice, but could always be isolated from the local lesion. The *Streptococcus mucosus*, on the other hand, was pathogenic, and found in the blood of the infected mouse; when, however, the pneumococcus invaded the mouth, the inoculated mice died of a pure pneumococcus septicæmia (so far as could be determined from smears on serum plates), while the *Streptococcus mucosus* could be recovered from the local lesion or from direct smears of saliva on serum-agar plates.

One other series of examinations is of interest in connection with the persistence of certain types of organisms in the mouth. In this case the mice throughout the entire period of examination invariably died from a septicæmia caused by a Gram-negative, capsulated bacillus of the Friedländer type. The first saliva was tested December 1, 1904, the last on April 28, 1905,—a period of six months. No pneumococcus was ever isolated from this person, but in smear plates from the saliva on serum-agar a Gram-positive, capsulated coccus, displaying the characteristic growth appearance of *Streptococcus mucosus*, was observed.

These observations suggested to the writer the probability, mentioned before, of such organisms being permanent residents of the protected salivary ducts or the tonsillar crypts, or possibly being accounted for by their connection with slight suppurative processes of the teeth and gums.

SUMMARY AND CONCLUSIONS.

The work carried on under the auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases, at the Bacteriological Laboratory of the College of Physicians and Surgeons, Columbia University, has consisted principally of a comparative study of the morphology, growth characters, fermentative activities, and agglutination reactions of pneumococci and allied organisms isolated by ourselves and the various workers under the Commission.

These organisms were from two chief sources: (a) from the

mouth and naso-pharynx of supposedly normal persons and persons suffering from minor inflammations of the naso-pharynx, and (b) from definitely pathologic sources, such as pneumonic sputum, pneumonic lungs, empyæma, the circulating blood of pneumonia patients, septicæmias, meningitis, and various minor lesions, usually due to infection with pneumococci.

One of the principal objects of the study was to make a careful comparison, in the light of the most recent knowledge of the biology of the pneumococcus and by the aid of the latest biological and technical methods, of the series of organisms from these two sources, and thus to determine definitely the true nature of pneumococcus-like organisms occurring in the mouths of normal persons.

The second and equally important object, ultimately dependent, however, upon the solution of the first, was the determination of the frequency of occurrence of typical pneumococci in the mouths of healthy individuals.

The investigations detailed in the present paper have dealt chiefly with the first problem and incidentally with the second, and lead to the following conclusions:

(a) That organisms, not to be distinguished by morphological characters or by any physiological peculiarities from true pneumococci derived from pathologic sources, occur with frequency in the mouths of healthy persons and those suffering from slight inflammations of the naso-pharynx, and that the only permissible and legitimate conclusion is that these organisms are true pneumococci.

(b) That there are other organisms in normal mouths and from pathologic sources that morphologically or by staining reactions are not definitely to be distinguished from pneumococci, and can only be recognized by a careful study of their fermentative activities and agglutination reactions. These organisms are non-inulin fermenters.

(c) That the organism known as *Streptococcus mucosus* is at times found in cultivations from the mouths of apparently healthy individuals, and that, although it shows certain peculiarities distinguishing it from the typical pneumococcus, it is probably very closely related to, and a variety of, this species.

(d) That other organisms occur which in their fermentations are indistinguishable from pneumococci, but which either morphologically or in agglutination reactions show a variation from this type. Some of these are probably temporarily or permanently modified pneumococci or *Streptococcus mucosus*; others, it may be, are streptococci of types which it has not heretofore been possible to recognize and describe. Some of these organisms were isolated from pneumonic lungs at autopsy, or from some internal source such as the circulating blood, and presumably have long resided under conditions which may be considered adverse, thus bringing about a modification of their morphology or physiology. This is supported by the observation that pneumococci from such sources—perfectly typical morphologically and in fermentative activities—are apt to show a lessening of their ability to agglutinate.

In connection with the other problem, the frequency of occurrence of true pneumococci in the mouth of healthy persons, our own investigations, limited to the study of the mouths of twenty-two individuals, have given the following:

In a series of fifteen persons from each of whom one specimen of saliva only was examined, typical pneumococci were found in seven out of the fifteen specimens, i.e., 46.6 %. In the case of the remaining seven individuals of our series, repeated tests, extending over weeks and months, were made, and the pneumococcus was demonstrated upon one or more occasions in the saliva of six out of the seven, i.e., 85.7 %.

It seems, therefore, more than probable that practically every individual, at least during the winter season, when exposed to environmental conditions such as those existing in New York City, acts as the host at some time or other, and probably at repeated intervals, of organisms of the true pneumococcus type.

None of the supposedly normal individuals examined by us had had a recognized pneumococcus infection or "cold" within a recent time, nor, with two well-marked exceptions, did any symptoms of infection develop in those whose mouths were found to contain pneumococci.

These two exceptions, detailed in an earlier section, strongly

suggest the etiological relations of pneumococci to some, at least, of the "common colds."

In conclusion, it is a pleasure to thank the various investigators connected with the work of the Commission, for their courtesy in furnishing us with the cultures, upon a study of which any success our work may have attained has so largely depended.

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EXPLANATION OF THE TABLE.

The table contains a list of the organisms examined, and an outline record of the results of the morphological examinations and of the principal fermentation and agglutination tests. Where two descriptions occur under the same heading, as in the morphology and inulin fermentation columns, the first description is the one reported to us, the second is the result of our own tests.

The sera used for the agglutination tests were obtained from immunized rabbits.

The agglutination tests, recorded in the pneumococcus immune serum column, were not all made in the same serum. The letter preceding the numerals indicates the special serum in which the test was made. The agglutination of the homologous organisms in these were as follows: a—400-800; b—400-800; c—200-800; d—200-400.

The results given in the column should not, therefore, be compared with each other, except when preceded by the same letter, but with the agglutination of the homologous organism.

The other agglutination tests were made in sera from *Streptococcus pyogenes* and *Streptococcus mucosus* immune rabbits, and in the case of sera "S. 7" and "CD No. 3" from rabbits immunized against organisms which fermented inulin but were atypical morphologically and did not agglutinate to an appreciable degree in pneumococcus immune sera. The P. & S. "8" serum was from a rabbit immunized against P. & S. culture "8," which is a non-inulin-fermenting capsulated streptococcus.

When two numbers are used to record the results of an agglutination test, the first indicates the highest dilution of the serum in which a complete precipitation of the agglutinated organisms occurred, and the second the highest reading with a hand lens.

All agglutination tests were made in small test-tubes by the macroscopic method, and careful comparisons were always made with the normal rabbit-serum and salt-solution controls.

No.	Morphology.		Inulin.		Pathogenicity.	Source.	Agglutinations.					Remarks.	
	Reported.	Central Lab.	Reported.	Central Lab.			"S. 7."	"C D 3."	S. pyogenes.	P. & S. "g."			
A 1	Typ.	Typ.	+	Acid	+	Saliva, normal.	b10-200		-		10-50	-	No sugars fermented; milk slightly acid. Glyco. not fermented.
" 2	Spher. dip.	Spher. dip.	-	-	+	Saliva, normal.	20-20						
" 3	Strepto.	Strepto.	-	-	+	Saliva, normal.	a-		-				
" 5	Spher. dip.	Spher. dip.	-	-	-	Saliva, normal.	b50		50		10-50	50	
" 6	Typ.	Typ.	+	+	+	Blood, pneumonia.	b100-400						Involution forms marked; most fermentations slow. May be S. mucosus.
" 7	Typ.	Typ.	+	+	+	Lung, pneumonia.	b50		10-50		0-50	0-20	
" 8	Typ.	Typ.	+	+	+	Meninges, meningitis.	a400-800		50			0-50	
" 9	Typ.	Typ.	+	+	+	Pleura, pneumonia.	b spontaneous; all same.						
" 10	Typ.	Spher. dip.	?	-	+	Lung, pneumonia.	b200-800						Involution forms marked; most fermentations slow. May be S. mucosus.
" 11	Typ.	Typ.	+	+	+	Lung, pneumonia.	b100-800						
" 12	Typ.	Typ.	+	+	+	Pus, empyema.	a10-50						
" 13	Typ.	Typ.	-	Acid	+	Pus, empyema.	a10-50						
" 14	Typ. caps.?	Typ.	+	+	-	Lung, pneumonia.	a200-800						Involution forms marked; most fermentations slow. May be S. mucosus.
" 15	Typ.	Typ.	+	+	+	Pus, acute otitis media.	a10						
" 16	Typ.	Typ.	+	+	+	Lung, pneumonia.	a200-400						
" 18	Typ.	Typ.	+	+	+	Saliva, normal.	b400-800						
" 19	Typ.	Spher. dip.	+	+	+	Saliva, normal.	a10-50		-				Involution forms marked; most fermentations slow. May be S. mucosus.
" 20	Typ.	Typ. caps.?	+	+	+	Pus, empyema.	a-		100-800			0-50	
" 21	Typ.	Gen. strep. to morph.	+	-	+	Pharynx, abscess.	b-					0-20	
" 22	Typ.	Typ.	+	+	+	Saliva, normal.	b200-800						
" 22a	Typ.	Typ.	+	+	+	Saliva, pneumonia.	a200-1600						Orange color in puncture.
" 23	Typ.	Atypical.	+	+	+	Saliva, normal.	b20-400						
" 24	Typ. no caps.	Strept.	+	-	+	Saliva, pharyngitis.	b-						
" 25	Typ.	Spher. dip. no caps.	+	+	+	Saliva, normal.	b-		20-100				
" 26	Typ.	Typ.	+	+	+	Saliva, normal.	b-800						Orange color in puncture.
" 27	Typ.	Typ.	+	+	+	Saliva, normal.	b0-20						
" 28	Typ.	Typ.	+	+	+	Saliva, normal.	b200-800						
" 29	Typ.	Spher. dip.	+	+	+	Spinal fluid, meningitis.	a200-800						
" 30	Typ.	Slt. atyp.	+	+	+	Lung, pneumonia.	a10-200						Orange color in puncture.
" 31	Typ.	Typ.	+	Acid	+	Saliva, pneumonia.	No test.						
" 32	Typ.	Typ.	+	+	+	Saliva, normal.	a0-50						
" 33	Typ.	Typ.	+	+	+	Saliva, normal.	a200-800						
" 34	Typ.	Typ.	+	+	+	Heart, acute endocarditis.	a100-800						

No.	Morphology.		Inulin.		Pathogenicity.	Source.	Agglutinations.						Remarks.
	Reported.	Central Lab.	Reported.	Central Lab.			Pneumococcus.	S. mucosa.	"S. 7."	"C D 3."	S. pyogenes.	P. & S. "g."	
A 35	Typ.	Typ.	+	+	+	Saliva, normal	2400-800	0-20			100	0-20	No sugars fermented; milk slightly acid.
" 36	Spher. dip.	Spher. dip.	-	-	-	Blood, mastoiditis.	210-20						Do.
" 37	Spher. dip.	Spher. dip.; narrow caps.	-	-	-	Pus; hip joint.	a-						Orange color in punct. Glycogen not fermented. Do.
" 38	Spher. dip.	Spher. dip.	-	-	+	Peritoneum; typhoid.	a-						Do.
" 39	Spher. dip.	Spher. dip.	-	-	-	Blood, puerperal sepsis.	a-						No sugars fermented; milk slightly acid.
" 40	Coccus.	Strepto. morph.; caps.	-	-	-	Lung, pneumonia.	a-						Do.
" 41	Spher. dip.	Spher. dip.	-	-	-	Pus, abscess.	20-20						No sugars fermented; milk slightly acid.
" 42	Spher. dip.	Spher. dip.	-	-	+	Lung, consolidated.	20-20		-	-			Orange color in punct.; glyco. and lactose fermented; milk not acid.
" 43	Spher. dip.	Mixed morph.	-	-	-	Lung, consolidated.	a-		-	-			Glyco. not fermented.
" 44	Spher. dip.	Spher. dip.	-	-	-	Lung, consolidated.	a-						Dextrose fermented.
" 45	Spher. dip.; caps.?	Lance. dip.; caps.?	-	-	+	Saliva, slight coryza.	a-		10-50	-			Glyco. not fermented.
" 46	Typ.	Typ.	+	+	+	Saliva, slight coryza	2400	-	50-400	-		0-50	
" 47	Spher. dip.	Spher. dip.; caps.?	-	-	-	Blood, empyema.	a-						
" 48	Spher. dip.	Biscuit dip.	-	-	+	Saliva, pneu. 4 mos. ago.	a-		100-800	-		0-1/10	No sugars fermented; milk slightly acid.
" 49	Spher. dip.	Spher. dip.	-	-	-	Lung, pneumonia.	a—all clear	Same.	Same.	-			Glyco. not fermented.
" 50	Typ.	Gen. strepto.	+	-	+	Saliva, normal	a-		100-200	-		0-20	
" 51	Typ.	Mixed	-	+	+	Discharge, coryza.	a-		20-800	-			Glyco. not fermented.
" 53	Spher. and typ.; no caps.	Typ. morph.	-	-	+	Saliva, normal.	a-						Glyco. not fermented.
" 54	Spher. and typ.	Mixed morph.	-	-	-	Saliva, pharyngitis.	20-50		20-50	20-50		0-20	Lact. and dextrose fermented; milk coag.
" 55	Spher. and typ.	Gen. spher.; narrow caps.	-	-	-	Saliva, normal.	a—spontaneous.	Same.	Same.			Same.	Dextrose and malt fermented; milk slightly acid.
" 56	Typ.	Typ.	+	+	+	Saliva normal.	2500-800						

A 58a	Typ.; no caps.	Mixed morph.; narrow caps.	-	-	-	Saliva, pharyngitis; abscess, tooth.	a -	0-30	-	-	Glyco. not fermented.
" 58b	Spher. dip.	Spher. dip. caps.	-	-	+	Saliva, normal.	No test.				Glyco. not fermented. Orange color in punct.; glyco. not fermented.
" 59	Spher. dip. and typ.	Spher. dip.	+	-	+	Saliva, normal.	a -				
" 60	Spher. dip. caps.	Bia. dip.; caps.	-	-	+	Saliva, normal.	20-10	-			
" 61	Spher. dip.	Lance. and becl. caps.; forma; caps.	-	-	+	Saliva, normal.	20-10	0-20			
C D 1	Typ.	Mixed morph.; caps.	+	+	-	Lung, pneumonia.	b -	0-30			
" 2	Typ.	Atyp.; narrow caps.	+	+	-	Lung, pneumonia.	b -	0-30			
" 3	Typ.	Lance.; narrow caps.	+	+	-	Lung, pneumonia.	b -	20			
" 4	Typ.	Coc.	+	+	-	Lung, pneumonia.	b -	20			
" 5	Typ.	Atyp.	+	+	-	Lung, broncho-pneu.	b -	100-200			
" 6	Typ.	Atyp.	+	+	-	Lung, pneumonia.	b -	100-200			
" 7	Typ.	Atyp.	+	+	-	Lung, pneumonia.	b -	0-30			
" 8	Typ.	Atyp.	+	+	-	Lung, pneumonia.	b -	100-200			
" 9	Typ.	Spher. dip.	+	+	-	Lung, pneumonia.	b -	100-200			
" 10	Typ.	Atyp.	+	+	-	Blood, pneumonia.	b -	100-200			
" 11	Typ.	Atyp.	+	+	-	Lung, pneumonia.	b -	0-30			
" 12	Typ.	Spher. dip.	+	+	-	Lung, pneumonia.	20	20-30			No sugars fermented; milk slightly acid.
" 13	Typ.	Spher. dip.	+	+	-	Saliva, normal?	a -	-			
" 14	Typ.	Spher. dip.	+	+	-	Lung, pneumonia.	a -	-			No sugars fermented; milk acid.
" 15	Typ.	Spher. coc.	+	+	-	Lung, pneumonia.	a -	0-30			No sugars fermented; milk not acid.
" 16	Typ.	Spher. dip.	+	+	-	Lung, pneumonia.	20-30	100			
" 17	Typ.	Sl. lanceo- late; no caps.	+	+	-	Abscess, shoulder.	a -	0-30			
" 18	Typ.	Spher. and typ.; caps.	+	+	-	Spinal fluid.	a -	0			
E 1	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pneumonia.	b100-800				
" 2	Typ.	Typ.	+	+	+	Mouth, saliva, swab, diph.	No test.				
" 3	Typ.	Typ.	+	+	+	Mouth, saliva, swab, bronchitis.	b200-800				
" 4	Typ.	Typ.	+	+	+	Mouth, saliva, swab, grippé.	b100-800				
" 5	Typ.	Typ.	+	+	+	Mouth, saliva, swab, normal.	b200				

No.	Morphology.		Inulin.		Patho- genicity.	Source.	Pneumo- coccus.	S. mu- cosus.	Agglutinations.				Remarks.
	Reported.	Central Lab.	Reported.	Central Lab.					"S. 7."	"C D 3."	S. pyo- genes.	P. & S. "8."	
E 6	Typ.	Typ.	+	+	-	Throat, swab, slight coryza.	b100-200						
" 7	Typ.	Typ.	+	+	-	Throat, saliva, swab, pneumonia.	No test.						
" 8	Typ.	Typ.	+	+	+	Throat, saliva, swab, pneumonia.	b100-800						
" 9	Typ.	Typ.	+	+	+	Throat, saliva, swab, pneumonia.	No test.						
" 10	Typ.	Typ.	+	+	-	Throat, saliva, swab, empyema.	b100						
" 11	Typ.	Typ.	+	+	+	Throat, saliva, swab, pneumonia.	b200-800						
" 12	Typ.	Typ.	+	+	+	Throat, saliva, swab, mouth normal, chronic neph.	b200-800						
" 13	Typ.	Small lance.; irreg. groups; no caps.	+	+	+	Throat, saliva, swab, normal.	b200-800						
" 14	Typ.	Typ.	+	+	+	Throat, saliva, swab, pneumonia.	b10-20						Controls same.
" 15	Typ.	Atyp.; nar- row caps.	+	+	+	Throat, saliva, swab, mouth normal, leukemia.	b-						
" 16	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pharyngitis.	b100-800						
" 17	Typ.	Typ.	+	+	+	Mouth, saliva, swab, typhoid.	b200-800						
" 18	Typ.	Typ.	+	+	+	Mouth, saliva, swab, mouth normal, cirrhosis.	No test.						
" 19	Typ.	Typ.	+	+	-	Mouth, saliva, swab, pharyngitis.	100						
" 20	Typ.	Pn. morph.; no caps.	+	+	+	Mouth, saliva, swab, pharyngitis.	b200-800					10	800-800
" 21	Typ.	Typ.	+	+	+	Handkerchief, pha- ryngitis.	-						
" 22	Typ.	Atyp.	+	+	+	Mouth, saliva, swab, pneumonia.	b200-800						
" 23	Typ.	Small lance.; irreg. groups; no caps.	+	+	-	Lip of cup after drinking; pharyn- gitis.	-?	-	10?	-	-	-	-
" 24	Typ.	Small lance.; irreg. groups; no caps.	+	+	+	Mouth, swab, pneu- monia.	-?	-		0	-	-	-

	Typ.	Strept.	+	+	+	+	+	10-100	90-100	50-100	0-50	50	
" 24	Typ.	Strept.	+	+	+	+	+						
" 25	Typ.	Typ.	+	+	+	+	+						
" 26	Typ.	Atyp.	+	+	+	+	+						
" 27	Typ.	Typ.	+	+	+	+	+	10-100				-	
" 28	Typ.	Typ.	Acid.	+	+	+	+	b100					
" 29	Typ.	Spher. dip.	+	+	+	+	+	b0-1/10	10-100				
" 30	S. m.	S. m.	+	+	+	+	+	b-					S. mucosus.
" 31	Spher. dip.; narrow caps.	Strept.; narrow caps.	+	-	-	-	-	b-	10-20			-	
" 32	Typ.	Typ.	+	+	+	+	+	a200-800					
" 33	Typ.	Typ.	+	+	+	+	+	a100-400					
" 34	Typ.	Typ.	+	+	+	+	+	a200-800					
" 35	Typ.	Typ.	+	+	+	+	+	b100-400					
" 36	Typ.	Typ.	+	+	+	+	+	b100-400					
" 37	Typ.	Typ.	+	+	+	+	+	a200-800					
" 38	Typ.	Typ.	+	+	+	+	+	No test.					
" 39	Typ.	Typ.	+	+	+	+	+	b100-400					
" 40	Strept.	Atyp.	+	-	-	-	-	a20-100					Glyco. not fermented.
" 41	Typ.	Spher. dip.	+	-	-	-	-	b-				-	Said to be S. mucosus.
" 42	Spher. dip.; caps.	Strept. morph. Spher. dip.	?	-	-	-	-	b-	0-20			-	
" 43				+	+	+	+	b20-100	0-20			-	
" 44	Typ.	Typ.	+	+	+	+	+	b200-400				-	
" 44A	Typ.	Atyp. morph.	+	+	+	+	+	a100-400				-	
" 45	Typ.	Typ.	+	+	+	+	+	cs200-400					
" 46	Typ.	Typ.	+	+	+	+	+						

Morphology.			Inulin.		Patho- genicity.	Source.	Agglutinations.					Remarks.
Reported.	Central Lab.	Reported.	Central Lab.	Pneumo- coccus.			S. mu- cousa.	"S. 7."	"C D 3."	S. py- ogenes.	P. & S. "8."	
E 47	Typ.	Small lance.; no caps. Typ. mor.;	+	-	-	Mouth, saliva, mouth normal.	c-	0-20	-	-	-	
" 50	Typ.	Typ. mor.;	+	+	+	Mouth, saliva, swab, pneumonia.	c100-400					
" 51	Typ.	Typ. mor.;	+	+	+	Mouth, saliva, swab, pharyngitis.	c100-400					
" 52	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pharyngitis.	s800					
" 53	Typ.	Typ.	+	+	+	Mouth, saliva, swab, mouth normal, ap- pendicitis.	s200-400					
" 54	Typ.	Typ.	+	+	-	Mouth, saliva, swab, mouth normal, bad heart.	c100-400					
" 55	Typ.	Typ.	+	+	+	Mouth, saliva, swab, mouth normal, rheumatism.	s100-400	20-100	20-100	100		
S. M. Bl. "S. 7."		S. m. Dip. and cl.; nar. caps. at times.		+			20-10 20-10	200-800	-	-	-	S. mucosa.
C 1	Typ.	Typ. caps.?	+	+	+	Sputum, acute ton- sillitis.	b50-200					
T 3	Typ.	Typ.	+	+	+	Sputum, tubercu- losis.	b50-800					
N 5	Typ.	Typ.	+	+	+	Throat, normal.	c200-800					
" 6	Typ.	Typ.	+	+	+	Throat, normal.	No test.					
" 7	Typ.	Typ.	+	+	+	Throat, normal.	c100-400					
" 9	Typ.	Typ.	+	+	+	Throat, normal.	c10-100				10-30	
" 10	Typ.	Typ.	+	+	+	Throat, normal.	No test.					
" 11	Typ.	Coc.	+	-	+	Throat, normal.	c-					
" 12	Typ.	Typ.	+	+	+	Throat, normal.	bo-200					
" 13	Typ.	Atyp.	+	+	+	Throat, normal.	c200-800					
" 15	Typ.	Typ.	+	+	+	Throat, normal.	co-200					
" 17	Typ.	Typ.	+	+	+	Throat, normal.	c200-800					
" 23	Typ.	Typ.	+	+	+	Throat, pertussis.	c100-400					
" 36	Typ.	Typ.	+	+	+	Sputum, pneumonia.	b200-800					
" 40	Typ.	Typ.	+	+	+	Throat, normal.	s100-200					
" 41	Typ.	Typ.	+	+	+	Throat, normal.	b200-800					
" 43	Typ.	Spher. dip.	+	+	+	Throat, normal.	bo-10	100				
" 44	Typ.	Lancet.	+	-	-	Throat, slight cold.	bo	10-20	-	-	-	
" 45	Typ.	morph.; caps. (?) Spher dip.	+	-	-	Throat, slight cold.	bo-10	0-20	10-30	-	-	

No.	Morphology.		Inulin.		Patho- genicity.	Source.	Agglutinations.						Remarks.
	Reported.	Central Lab.	Reported.	Central Lab.			Pneumo- coccus.	S. mu- cosus.	"S. 7."	"C D 3."	S. py- ogenes.	P. & S. "8."	
Z 1	Typ.			+		Blood, pneumonia.	350-200		0-20	0-10			Probably <i>M. tetra-</i> genus. Many involution forms.
" 2	Typ.			+		Sputum, pneumonia.	20-10		—	—			
" 3	Typ.			+		Blood, pneumonia.	20-10		0-20	0-10			
" 4	Bis. dip.			—		Sore throat.			Same as Pn.	Same as Pn.			
" 5	Strept.			—		Throat, normal.	a small clumps in all.						
" 7	Typ.			+		Lung, pneumonia.	1100-400						Many involution forms.
" 8	Typ.			+		Blood, pneumonia.	1100-400						
" 9	Typ.			+		Blood, pneumonia.	a sponta- neous.	10-100	Clumps in all.	Same.			
" 10	Spher. and typ.			+		Sputum, pneumonia.	2400-800						
P. & S.													
1	Typ.			+		Sputum, pneumonia.	1200-800	200-800	50-400	—	0-100	—	S. mucosus.
2	Typ.			+		Sputum, pneumonia.	No test.	200-800		—	0-100	—	
3	Typ.			+		Sputum, pneumonia.	1400-800						
4	Typ.			+		Eye, keratohypopyon.	No test.						
5	Typ.			+		Sputum, pneumonia.	No test.						
6	Typ.			+		Sputum, pneumonia.	No test.						S. m. from same mouth as No. 7.
7	S. m.			+		Sputum, pneumonia.	a—	10-200	—	—	0-20	—	
8	Gen.			+		Saliva, normal.	a—	—	50-100	—	0-20	50-100	
9	strept.; caps.			+		Saliva, normal.							
10	Typ.			+	Acid.	Saliva, normal.	1400-800						
11	Typ.			+		Saliva, alt. "cold."	No test.						S. m. from same mouth as No. 7.
12	Typ.			+		Saliva, normal.	1200-400						
13	Typ.			+		Saliva, slight catarrh.	?						
14	Typ.			+		Saliva, normal.	d100-200						
15	Typ.			+		Saliva, normal.	d—						
16	Spher. dip.			+		Saliva, normal.	No test.						S. m. from same mouth as No. 7.
17	Typ.			+		Saliva, normal.	b0-10	10-50	—				
18	S. m.			+		Saliva, normal.		100-200	50-400	0-20	100		
19	Typ.			+		Saliva, normal.	1100-800						
20	Typ.			+		Saliva, normal.	b400-800						
21	Coc.			—		Saliva, normal.	No test.						S. m. from same mouth as No. 7.
22	Spher. dip.			—		Saliva, normal.	b20-50	10-20					
23	S. m.			—		Saliva, normal.	b0-10						
24	Typ.			—		Saliva, normal.	b—						
25	Typ.			—		Pleural fluid, pneu.	b20-200						
26	Typ.			+	Acid.	Saliva, normal.	b800						S. m. from same mouth as No. 7.
27	Typ.			+		Saliva, normal.	b—						
28	Atyp.			—		Saliva, normal.							
29													
30													
31													S. m. from same mouth as No. 7.
32													
33													
34													

A NOTE ON THE ELECTRIC CONDUCTIVITY OF BLOOD DURING COAGULATION.

BY ROBERT T. FRANK.

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Columbia University.]*

THE methods now in use for determining the coagulation time of the blood leave much to be desired. The most satisfactory apparatus is that of Brodie and Russell,¹ which takes advantage of the fact that the red blood cells of a drop of blood, when agitated by an air current, revolve uniformly until fibrin threads begin to form.

The present study was undertaken in the hope of elaborating a new method, which it was thought might serve to measure accurately the combination of physical and chemical changes occurring during clotting. This expectation has not been fulfilled, but the facts determined during the course of the work, though negative, were considered of sufficient interest to be recorded.²

The theoretical considerations were based upon certain properties possessed by substances in the colloidal form, especially in their relation to electrical conductivity. Fibrinogen, as found in the fluid blood, is a colloid, forming part of the other colloidal substances in the blood (albumens, globulins). Sjöqvist,³ and Bugarszky and Tangl⁴ have shown that albumens, when added to dissociable electrolytic solutions, reduce their conductivity. Blood is such a mixture,

¹ BRODIE and RUSSELL: *Journal of physiology*, 1897, xxi, p. 403.

² The experiments were performed during the winter of 1904. In the March number of this journal, 1905, appears an article by T. M. WILSON, *Measurement of electrical conductivity for clinical purposes*, in which the author has tried a similar experiment, though only a single determination was made. The result is identical with mine. I desire to thank Prof. JOHN G. CURTIS, of the department of physiology, and Profs. PRUDDEN and WOOD, of the pathological department, for many courtesies extended to me.

³ SJÖQVIST: *Skandinavisches Archiv für Physiologie*, 1895, v, p. 277.

⁴ BUGARSKY and TANGL: *Archiv für die gesammte Physiologie*, 1898, lxxii, p. 531.

for it contains numerous electrolytes (carbonates, chlorides, sulphates of sodium, potassium, calcium, etc.) in solution, and the above-mentioned colloids. Besides the dissolved constituents the blood contains solid particles (the red and white cells) in suspension.

Oker Blum¹ found that the resistance of serum derived from coagulated blood was regularly greater than that derived from defibrinated blood. G. N. Stewart² determined that the resistance of defibrinated blood is 3-5 times greater than that of serum; also that the settling of the blood corpuscles in defibrinated blood increases the resistance of the lower layers, because the corpuscles act as foreign, non-electrolytic bodies in suspension (Oker Blum).³ This increase, due to settling of the corpuscles, can be neglected if the observations occupy but a short time.

The first step of the writer's research was to determine whether during the process of coagulation any change of conductivity would result, especially if the clot were left *in situ*. It might be expected that the change from the colloidal fibrinogen to the solid fibrin would be accompanied by a change in conductivity. What form this change might take could not be predicted, as several as yet unstudied factors require consideration.

As already stated, fibrinogen would act as a partial insulator. Actual experiment alone could show whether changing this substance into fibrin would increase the conductivity, or whether the new-formed fibrin, acting as a solid, would in its turn offer a greater obstruction to the current, for the above-mentioned results of Stewart and Oker Blum throw no light upon this. The small amount of calcium regularly removed from the solution (probably carried down mechanically) and incorporated in the clot, would likewise tend to increase the electrical resistance by reducing the amount of electrolytic substance in solution.

The few investigations at all resembling those to be described are those of Van der Laan,⁴ dealing with the changes occurring in milk through acidification, or those of Buchinger,⁵ which deal with similar changes brought about by pepsin, in which again the forma-

¹ OKER BLUM: See Hamburger, Osmotischer Druck und Ionen-Lehre, 1904. i, p. 477.

² G. N. STEWART: Journal of the Boston Society of Medical Sciences, 1897, i, No. 16, p. 18.

³ OKER BLUM: Archiv für die gesammte Physiologie, 1900, lxxix, p. 501.

⁴ VAN DER LAAN: Inaugural dissertation, Utrecht, 1896.

⁵ BUCHINGER: Inaugural dissertation, Giessen, 1902.

tion of acids must be reckoned with; and those of Bredig¹ and Lottermoser,² etc., who devised the preparation and studied the properties (among them the conductivity) of the metallic colloids and their insoluble hydrosols. Their results cannot be transferred or used in this connection.

In the writer's experiments the electrical conductivity was determined by the method of Kohlrausch³ and his smaller apparatus, with sliding bridge, was employed. Freshly drawn blood was caught in small vessels, paraffined to retard coagulation, and kept at constant body temperature until clotting was completed. The conductivity was determined at frequent intervals during the entire process. Electrodes of 2-3 sq. cm. surface, heavily platinized, were used. They were separated between 1-2 cm., depending upon the amount of blood obtainable.

The result showed that there *was no constant or appreciable change during coagulation*. This held good when the electrodes were placed at the uppermost level of the fluid or lower, where the well-formed clot interposed. The fundamental points of the study having thus proven negative, further experiments were not undertaken.

Whether the amount of fibrinogen in blood is too small to show recordable changes with the apparatus used, or whether (which is unlikely) the numerous chemical and physical changes occurring in the complex process of coagulation, exactly neutralize each other, could not be determined.

It might prove of interest to try similar experiments with simpler fluids containing fibrinogen in relatively larger quantities, such as hydrocele fluid or concentrated, artificially prepared solutions of fibrinogen.

¹ BREDIG: *Anorganische Fermente*, Leipzig, 1901.

² LOTTERMOSER: *Über anorganische Colloide*, Stuttgart, 1901.

³ KOHLRAUSCH und HOLBORN: *Das Leitvermögen der Elektrolyte insbesondere der Lösungen*, Leipzig, 1898.

CLEAN AIR.*

By T. MITCHELL PRUDDEN, M.D.,

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FROM the beginning to the end of his life three things man must have, whatever else he may lack. These are air, water, and food. Under natural conditions the air is everywhere abundant; the supply of water is bountiful or may be obtained in nearly all regions naturally fit for human habitation. Under almost all circumstances the consumer or somebody else must work for his food.

But the securing of these three essentials of life becomes more complex and difficult as men herd together in communities, and especially in great cities. The air gets contaminated by enclosure in the confined spaces in which we live. The water becomes scanty from the large local demands upon a limited supply, or unwholesome by its pollution through community waste. Food grows costly by the increase in population and the grouping of people who are not themselves food producers; it becomes deficient or harmful through the rapacity of its purveyors and the ignorance and intemperance of its consumers.

Of these three essentials to life it is the air alone which now concerns us. We need not dwell upon the harmful products of human waste in the air, nor upon the various other ways and forms of air contamination which involve its gaseous constituents. These have been the subject of extensive studies, and their nature and significance are well known. The common problems of ventilation find adequate treatment in the standard treatises on hygiene and sanitation.

But there is another kind of air contamination which has not hitherto received as much attention as its importance justifies, namely, floating dust, to which this paper is largely devoted.

Dust as an inciter of disease has long been recognized in occupations which involve the setting free in the air of large quantities of finely pulverized

*Read before the Practitioners' Society, January 5, 1906.

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material. Such trades as certain forms of mining, wool sorting, stone cutting the making of certain pigments and chemicals are placed in a class by themselves as especially dangerous occupations.

But it is comparatively recently that the significance of floating matter in the air in relation to the health of the people at large has been realized, and the accumulation of this new lore is closely linked with the development of our knowledge of bacteria and other minute infectious agents.

It was clear enough from the beginning to the devotees of the new science of bacteriology that among these minute commensals of man only a few were of sinister import. But to most physicians and to the public, bacteria were things to conjure with, and all shared in the bad repute of the few which were demonstrably pathogenic. So for a time the mere presence of many bacteria in food or water or air was looked upon as a serious menace.

But with the discovery of one after another of the inciting germs of various infectious diseases, a knowledge of their sources and the conditions under which they flourish or are destroyed, a more intelligent and discriminating viewpoint was reached. Men came to dread the forms which are harmful, and set about finding means to curtail their ravages, and left the remainder, the great majority, to their own devices, or made them the subject of curious and fruitful biologic study.

It soon became clear that the infectious microorganisms are conveyed from an individual harboring them, either directly by personal contact or indirectly through some contaminated intermediary, or through some solid or liquid used as food or drink, or, finally, through the air in the form of visible or invisible dust.

We leave, as not bearing directly upon our theme, the various modes of conveyance of infectious agents by immediate contact and through contaminated food and drink, and turn to aerial infection.

Infectious material, depending as it always does upon microorganisms of some sort, can only pass from one individual to another through the air as particulate bodies. Thus the study of the physical conditions under which fine particles can be disseminated in the air, how long they can remain in suspension under various conditions, and how these conditions affect the viability and virulence of infectious microorganisms, has become of great importance.

It was early learned that bacteria are not readily separated from fluids or moist surfaces by air currents, and that the exhaled breath is practically germ-free even in those suffering from communicable infectious diseases. It was found that many microbes are killed by drying, by sunlight, by immersion in water; or if not killed, many pathogenic forms are greatly reduced in virulence.

On the other hand, a host of careful studies has been made on the various safeguards of the body against the entrance of microorganisms, and a flood of light has been let in upon the subtle and highly effective processes through which invading germs of all degrees of virulence are safely disposed of within the deep recesses of the tissues, when by some mischance they have overcome the barriers.

The early conception of the risks of aerial infection is clearly exemplified by the story of the Lister spray and its various modifications, which at one time dominated the field of practical surgery. It was then the endeavor at all hazard to remove the possibility of bacteria of any kind which might be floating in the air, entering the open wound. It did not matter much where these came from. All bacteria looked alike to these early devotees to antiseptics.

But the remarkable capacity of the living tissues to kill and safely dispose of most bacteria soon impressed itself upon the surgeons through practical experience and the studies of the laboratories. They began to realize that the direct conveyance to the wound of a few forms of well-known germs from an outside source of infection is a far more serious menace than the indirect conveyance even of the same organisms which have been subjected to the unfavorable influences of drying, sunlight, etc., usually involved in the formation of dust. Thus it is that most surgeons to-day, having secured what they consider reasonable cleanliness in their operating rooms, disregard the air as a risk, and concentrate their attention upon direct sources of infection, such as hands, instruments, dressings, the seat of operation, etc.

Similarly, the alleged sources and frequency of aerial infection through the respiratory apparatus began to be accepted with more reserve as knowledge grew on the one hand about the nature and vulnerability of infective microorganisms and on the other about the self-protective capacities of the human and animal body. Finally, one of the most

significant features in infection secured recognition, namely, that the simple presence or growth of infective agents in the body does not suffice for the incitement of disease unless a series of favoring conditions of the body itself be present. This prerequisite to infection, sometimes very subtle, and often not yet at all clearly understood, is named pre-disposition or susceptibility, and its final recognition has contributed largely to the juster estimate of the common risks of infection, as well as to the brightening outlook for the suppression of many serious maladies.

Thus the drift of discovery and the perspective which our present outlooks furnish, lead to a more critical and a somewhat chastened estimate of the actual risks to which we are commonly exposed from floating matter in the air of inhabited regions.

Still, when all is said in favor of the saner view which the knowledge of to-day may justify, the proper disposal of infective secretion and the removal of dust which gathers in the close and often crowded spaces in which many pass a large share of their lives is of great importance, and its neglect has a direct bearing upon the increase in respiratory diseases which statistics show.

Let us therefore look more closely at some of the more recent studies on dust and its significance.

We seldom realize what an enormous number of dust particles may be floating in the air of enclosed places which in diffused light seems clear and pure. But let a beam of sunlight fall through a small opening into the air, or cast a powerful electric light across the dusty space. Then an incredible number of minute particles may be seen dancing about, forming indeed a sharp luminous pencil made up of the reflected rays thrown from each tiny mote.

How numerous these particles may be even in inhabited places, it is not easy to determine. But various methods have been devised for counting them.

Dust-containing air in measured volumes has been caused to bubble through fluids, the latter evaporated, and the particles weighed or counted. It has been filtered through cotton and the amount determined. It has been blown against glass plates covered with adhesive material, and thus an approximate estimate arrived at.

The most accurate and ingenious method of dust analysis is that of the English physicist Aitken, who has devised an instrument called the Aitken dust

counter, by which we may determine the exact number of particles in any given volume of air.

It is worth looking at for a moment, this ingenious instrument of the English scientist. It is well known that when the air is fully saturated with moisture and is then suddenly cooled, the watery vapor condenses as fog or rain. It is not so well known that in the formation of fog or rain a particle of floating matter in the air, that is, of dust, forms the nucleus upon which every drop or droplet is condensed. Now, it occurred to Aitken that if he could reproduce experimentally, in a measured volume of dusty air, the conditions under which fog forms, and then could count the fog droplets, he would know how many dust particles there were in the air; the counting of the fog droplets being easier than counting the dust particles, because they are much larger.

The "dust counter" as it was finally perfected consists of a small, accurately measured, air-tight chamber, connected with an air pump and with an arrangement for introducing small measured volumes of dusty air. The air chamber, saturated with moisture, is first charged with a measured amount of the air. Then with a sharp thrust of the air pump a partial vacuum is created in the chamber. The air expands, and as it does so suddenly cools, so that a fog droplet is formed around each floating dust particle. The operator is watching the illuminated interior of the chamber through a lens and can see the shining droplets as they suddenly flash into the field of vision and fall in a minute shower upon a ruled plate at the bottom of the chamber. Then, when all the fog droplets have fallen and the mimic shower is over, he counts the microscopic splashes, each one representing a whilom floating dust particle, and makes his simple calculation of the number which were in a thimbleful, or a breath, or a roomful of the dusty air. No matter how small the floating particles may be, the fog catches them every one.

I show to you Aitken's dust counter set up and ready for use.

Prof. Aitken has made many interesting studies of air under various conditions, and the revelations of his analyses are even more striking than those of the sunbeam, because they are expressed in figures. For example, he has estimated on the basis of counts with his instrument, that the devotee to the gentle cigarette may set free upon an unwilling environment at one full average exhalation, not less than four thousand million particles of smoke.

The number of dust particles in the air we breathe, even when very impure, is of course usually much less than this, and many factors must be taken into the account before one ventures upon a numerical statement of the average number in any given place. For example, the air out of doors may be largely cleared of dust by rain and fog. On the other hand, dry winds may sweep dust particles upward in vast numbers from the ground, and hold them suspended for hours upon the air, driven hither and thither in clouds of varying density. Again, the great volumes of relatively clean air moving across a dusty region may so dilute the impure air that the number of dust particles in a given volume is greatly reduced. Or they may be carried away altogether.

Finally, one of the most important of the physical agencies determining the number of dust particles at any fixed place is the gradual settling of the dust whenever the air is moving slowly or is still.

In his studies upon the floating matter in the air, Tyndall found that these myriad dust particles, if enclosed in a tube, soon settle upon its sides and bottom and the enclosed air becomes as clear and motionless as are the stellar spaces. This is, indeed, only a more refined form of the experiment which every housekeeper makes who closes the room after sweeping to allow the dust to settle.

Thus it is evident that when we are told that somebody has found so many thousands or millions or hundreds of millions of dust particles in the air of some town or street or building, we may justly insist upon knowing the conditions of his analysis before we can concede any special significance to his figures.

What the effect may be upon the health of persons who breathe this more or less dust-laden air can be more intelligently considered after we have glanced at one most significant form of dust, namely, that containing bacteria or other kinds of microorganisms.

Microorganisms floating in the air as dust are sometimes single, more often in small clusters or masses joined together as they have grown, and are very apt to be clinging to some other forms of dust particles.

The number of microorganisms which the air may contain varies, like other forms of dust, with varying conditions. In the case of these, as of other dust particles, the actual number determined by analysis

is of little value as expressing averages, unless the conditions are carefully recorded. In general it may be said that the out-of-doors air contains far less microorganisms than that in rooms where men and animals gather, because of the dilution constantly occurring from the vast volumes of clean air sweeping across the country.

There are two common methods of determining the number of living microorganisms in the air.

The first and most accurate is by allowing a measured volume of air to filter slowly through a tube of dry sterilized sand, which retains all the dust particles, microorganisms among the rest. The entire content of the tube is then planted in a suitable culture medium. The number of colonies of microorganisms which grow under these conditions is taken to indicate approximately the number of living germs in the measured air volume.

Here is the apparatus which we use for quantitative analysis of air. This is a vacuum cylinder so adjusted that when exhausted to a certain point on the gauge it will draw in ten liters of air, which passes through this tube partly filled with sterilized sand. Here is a culture plate after the operation, in which you see among the grains of sand the scattered colonies of various forms of microorganisms.

The second method of so-called biological air analysis consists in exposing culture plates of nutrient medium to the air for a definite time, and then counting the colonies which develop from the dust which has settled upon the surface during the exposure. Care is, of course, taken in this form of analysis to secure quiet places for the plate exposures, and the results, while not so accurate as by the first method, are still of value in comparative studies, provided the conditions of the observation are carefully noted. To record simply the numerical result of such an experiment is of little value, because the bacterial content of the air in still places is constantly diminishing by the settling of the dust particles.

Here is a series of culture plates which have been exposed for fixed periods to the falling dust of an inhabited room. You see the great variety of forms in the germs which have grown to colonies upon the surfaces.

But whether the air contains few or many living germs, their significance as regards the welfare of those who use it rests most directly upon the forms

which are capable of inciting infectious disease. The others are harmful in just such manner as all dust may be harmful when in the air we breathe.

As was natural, very careful search has been made in dwellings, public conveyances, places of public assembly, hospitals, etc., for living pathogenic microorganisms. And the booty has been considerable. Tubercle bacillus, pneumococcus, streptococcus, staphylococcus, diphtheria, anthrax, tetanus, are among those recorded.

But, as we shall presently see many of the pathogenic microorganisms which have been found in inhabited places have been gathered under conditions which did not indicate that they were in such a finely divided state that they could have contributed largely to the risks of dust infection through inspired air.

We may now concentrate our attention upon dust within doors since it is here that the most serious questions arise relating to the maintenance of health.

We shall first look at some of the physical conditions determining the distribution of microorganisms in the air as dust.

While it has long been known that dust particles settle in still air so that under experimental conditions the air of a small receiver may become quite dust-free, it was not clear that a similar condition could be secured with floating microorganisms in living-rooms and places of assembly, since here, though tightly closed, the air is always astir, sometimes with currents of considerable force.

But some years ago Stern found in a small, tightly-closed, empty room, whose air he had changed with certain easily recognized bacterial dust, that within from twenty to thirty minutes the larger part, in from one and one-half to three hours almost all the bacteria, had settled to the floor, so that the air was practically germ-free.

It had commonly been assumed that in ventilation the air renewal would suffice to remove not only the vitiated air, but its floating particles as well. So Stern studied the effect of ordinary ventilation on the dusty air of his experimental chamber. He found that with ventilation sufficiently effective to change the whole volume of air four times per hour there was little gain over the clearing of the air by simple settling. On renewing the air six to seven times an hour he found some gain; while if the whole were changed ten times per hour, there was a rapid removal of the bacteria from the room. It

should be remembered that under suitable conditions the air in a room may be renewed four or five times per hour without the creation of an appreciable draft. It is thus seen that in the ordinary effective ventilation of a room bacteria are not removed from the air to any considerable extent.

Another point of great interest is covered by the experiments of Stern. It has long been a popular impression that the opening of windows, especially when the wind is blowing, is effective in the removal of dust from rooms, even though this has already settled upon the open surfaces. Stern found that this is not the case. He studied the effect of air currents upon bacterial dust which had already settled naturally to the surfaces of floors, fabrics, etc., and found that even very rapid currents of air—six to eight feet per second—blown across such surfaces do not dislodge the bacterial dust in appreciable degree.

It follows from these experiments that the ordinary ventilation, however effective it may be in the supply of fresh air and the removal of gaseous contaminations, has no noteworthy effect in the removal of dust, either floating or at rest.

Indeed, if the fresh air introduced in artificial ventilation contains dust, with the slowly moving currents between the entrance and exits, the dust steadily falls, so that the rooms are actually settling basins in which dust, unless otherwise removed, continually accumulates.

These experiments show also that the hanging of garments out of doors in the air with the expectation that infective agents will forthwith abandon them, is not to be commended. For unless such articles be brushed, or beaten, or shaken, or flap violently in the wind, bacteria are not largely removed.

Although, as stated above, various pathogenic organisms have been found in the dust of inhabited places, the fact that a large proportion of most of them are killed by drying and by exposure to sunlight, as well as confidence in the effective safeguards of the normal body, led to a widespread disregard of the risks of dust infection except in a few instances, among which the most conspicuous is tuberculosis. The pulmonary forms of this disease so frequently involve the discharge of large numbers of living bacilli that this risk was recognized, while in other infectious diseases it has been largely ignored.

But some years ago, Flügge investigated the fresh

infective material which may be discharged from the mouth in speaking and coughing, and from the mouth and nose in sneezing. The results of these studies, carried on largely by himself and his assistants in Breslau, brought to the front a series of new and sinister possibilities of air infection. In brief, Flügge and his associates found that not only in sneezing and coughing, but also in ordinary speech, the secretions of the nose, mouth, and throat may be cast forth in considerable quantity for a distance of several feet, not only in the form of visible droplets, but as a more or less abundant, invisible spray, which may remain suspended for from half an hour to several hours, and may be carried for long distances on such slowly moving air currents as are common in inhabited rooms.

The method by which these results were obtained was simple. The mouth and throat of the subject was rinsed with a small amount of an emulsion of a harmless bacterium—frequently the *B. prodigiosus*—and he was placed in or in front of a small sterile cabinet, in various parts of which culture plates were exposed. At the end of the seance, after speaking or coughing or sneezing, the dust and invisible spray were permitted to settle on the culture plates, where later the colonies which had grown were counted.

There is much difference in individuals as to the extent of dispersal of material in speech, as was shown by declamation into the cabinet by various persons. It is rather sharpness than loudness in speech, and especially the enunciation of certain of the consonants, such as t, k, p, b, f, which give rise to the most generous ejection of the secretions. There is a marked difference in individuals who cough, depending in part upon the vigor of the act, the character of the secretions, and the position of the lips. It was found that most of all in sneezing the air may be widely contaminated.

Not only was it possible to infect culture plates with microorganisms taken into the mouth with experimental intent, but patients with tuberculosis were so placed as to cough and sneeze in enclosed places in which animals were confined, with the result of tuberculous infection of the exposed animals.

These studies of Flügge and his followers have thus shown, among other things, that in the immediate vicinity of persons whose mouths harbor pathogenic microorganisms there is a varying but often considerable risk of the direct conveyance, especially in coughing and sneezing, of fully virulent infectious

material, not only in the form of visible droplets, but as invisible spray.

Later studies of de Leon, Hamilton, and others, have emphasized the importance of this floating spray in the conveyance of streptococci and other pyogenic organisms from infected mouths during surgical operations, and the incitement of fresh infections in hospital wards and elsewhere.

While in general it may be said that the directly infectious zone does not usually reach beyond two or three feet, it is still to be remembered that so minute are the particles often expelled in enormous numbers in the commonly invisible spray of sneezing, that these may be carried for many feet on air currents as slow as those involved in ordinary ventilation or such as are invariably present in inhabited rooms.

Such minute particles, moreover, which harbor microorganisms not killed by drying, may ultimately form infectious dust so light that when wafted into the air by the movements of persons or by improper methods of cleaning, they may remain long suspended in suitable form and condition for inhalation.

The time during which bacterial and other dust remains suspended in the air is naturally dependent upon the weight of the particles, so that in forming an estimate of the risks of infection from floating dust, one has to consider not only the physical characters—size, weight, aggregation, etc.—of the various pathogenic microbes, but also the conditions under which they gain access to the air.

In this connection the critical studies of Flügge and his associates have brought to light some interesting points.

In the first place, it has become clear that not all the infectious material which may be cast out of the body as sputum is always readily and speedily transformed into dust. In the drying of the mucus with which much of this material is mingled, a process ordinarily rather slow, the bacteria are closely imprisoned so that only under certain conditions, such as the rubbing and beating of soiled clothing, the shuffle and tread of feet over contaminated floors, dry sweeping, and the like, is the excretion sufficiently comminuted to float in the air as dust. Even when this is the case, it has been found that a large part of such pulverized excreta is still in too large particles to remain long suspended on the air.

This condition of affairs was not appreciated in the earlier studies on dust infection, and in many

instances the material called dust, collected in various places for analysis, was evidently not in such form as would have remained long in suspension. Thus it is that while the earlier identification of various pathogenic microorganisms in the dust of rooms was correct, the inference as to the constant risk of dust infection in such places was frequently at least somewhat overestimated.

Many studies have been made on the transportation of dry germs in floating dust, especially indoors. Such air-borne germs readily pass from room to room on the ordinary air currents. If doors are closed, they make their way through cracks and keyholes. They are wafted from one story upward to the next, and readily find their way into closed wardrobes, bureau drawers, and between the leaves of open books.

The effect of walking over floors on which bacteria-laden dust has settled is to set large numbers astir, but in still air these do not commonly rise more than a few feet and then slowly settle back again. Dry sweeping of such floors sends clouds of germs into the air, contaminating it in every part.

We have thus seen that the amount of dust in the air which we breathe is not only constantly varying, but that its significance in relation to health is closely linked to the sources from which it is derived. Among the microorganisms which form a constant part of the dust in inhabited regions, it is those which are derived from men and animals, themselves the subjects of infection or harboring pathogenic microorganisms which are of dominant significance.

Let us now glance at some of the ways in which the body is protected against the entrance of dust and the means by which it is disposed of when it does pass, as is frequently the case, even the most effective barriers.

In normal breathing through the nose, the air impinging upon the moist surface of the tortuous passages loses a considerable part of its dust and bacteria. In the throat, larynx, and trachea, a similar clearing occurs, so that at the end of inspiration the air has been so far freed from its floating particles that it emerges in the expiration practically dust and germ-free.

If the nasal passages are not normal, or breathing through the mouth is practised, the air contaminations may pass more deeply into the recesses of the lungs, or may still remain to a certain extent suspended in the expired air.

Many of the dust particles which may lodge upon the walls of the deeper air passages are swept upward by the ceaseless ciliary movement and cast out, as are the secretions and dust accumulations from the nose and throat. The growth of many microorganisms is inhibited by the mucus of the air-passages. Many inert dust particles which are taken into the tissues at the tonsils or in the deeper recesses of the air tubes are carried in the lymphatics to places of temporary or permanent deposit. Many microorganisms within the tissues are destroyed by phagocytes or by the body fluids.

Thus it is that although large numbers of dust particles may enter the respiratory passages at every breath, the larger proportion are usually safely disposed of, most of them through the secretions of the throat and nose.

It has long been known that a certain number of inert dust particles from the inspired air, in spite of the tortuous and narrow passages which they must pass in the deeper recesses of the lungs, do reach the air vesicles, from which they may be largely removed to the adjacent tissues or to the nearest lymph nodes.

Whether living bacteria can reach the air vesicles under the ordinary conditions of respiration, is a question much discussed and has given rise to many painstaking researches. One group of workers has concluded that the lungs, except for the larger air passages, are usually germ-free. On the other hand, many have concluded from equally careful studies that the lungs of both men and animals often, if not always, contain a moderate number of living microorganisms. The technical difficulties in this research are so great and the conditions under which they have been undertaken are so various, that this difference of opinion is not surprising.

Without attempting to marshal the evidence adduced on both sides of the question it appears that in most of the earlier studies on the artificial introduction of living microorganisms into the lungs, the time which was allowed to elapse between the introduction of the germs and the examination of the lungs was too long, so that the possibility that the lungs might rapidly dispose of germs lodged in their deeper recesses was overlooked.

In fact, it has been found that in experiments in which animals are exposed to an atmosphere laden with bacteria-containing dust or spray, if the animals be killed at once after the exposure, living microorganisms may be found even in the deepest portions,

while if even a short time be allowed to elapse they will have entirely disappeared.

While, however, in the air passages and lungs we have a most efficient protective mechanism against the entrance and permanent lodgment under ordinary conditions of both inert dust particles and living microorganisms in such situations as would involve serious damage to these delicate organs, it should be remembered that the efficiency of these safeguards may be greatly diminished if they are overworked and abused.

The lungs of practically all persons who live indoors contain a considerable amount of soot, which blackens them. The irritation incited by this foreign stuff leads in most adults to some part of the lungs becoming dense and useless. The overworking of the delicate mechanism for filtering the foreign matter out of the lymph flowing from the lungs to the interior blood channels leads to serious damage of this mechanism and to an increased vulnerability to infection which the healthy body should and could resist.

These conditions resulting from excessive dust inhalation are not necessarily obvious through illness or apparent reduction in vigor. But in the long run, with many other offenses against personal hygiene, they are enough now and then to swing the balance toward disease and death.

There can be no doubt that various forms of catarrh of the nose and upper air passages, if not directly incited, are favored and perpetuated by the inspiration of dust-laden air.

Under these conditions, too, several forms of disease-producing bacteria find favorable conditions for continuous growth, while the discharges from such infected regions afford a perpetual source of widespread distribution.

It is not necessary for us to enter into the question much discussed of late, whether infective bacteria which enter the body by the inspired air commonly reach the lungs directly through the air passages, or whether in many instances they may not enter through the tonsils or the gastrointestinal canal, reaching the lungs by way of the blood or lymph vessels. The necessities for cleanliness in the air are the same in either case.

Perhaps the most common of the pathogenic microorganisms to be widely dispersed in the air as floating particles are the streptococcus and staphylococcus, which are apparently frequently concerned in

the incitement of colds and of the lamentably frequent catarrhs. So general is the dispersal of these organisms and of those inciting influenza and pneumonia, that they are now generally recognized as regular denizens of the mouths of dwellers in towns.

The experience of those who come home to the cities after a summer of the most varied exposures in germ-free air without colds or other disturbances of the respiratory apparatus, only to inaugurate the winter with a bronchitis or influenza, is evidence of the vicious conditions in public conveyances and places of assembly.

Some slight exposure to draughts, overexertion, indiscretion in eating, and various other slight departures from healthful conditions, apparently suffice when these germs are present in virulent form, to precipitate an illness which often shadows the winter if it does not lead to serious or fatal disease.

It is clear to-day that in one group of infections, of which typhoid fever and cholera may be taken as types, the risks of the transmission of the infective material in floating dust are slight. In another group in which direct contact of vulnerable surfaces with infective secretions or exudates is requisite for communicability, such as syphilis, most wound infections etc., aerial infection may be left largely out of account.

On the other hand, in the exanthemata we have the clearest examples of aerial transmission of the infective agents, though these are as yet unknown and can be guarded against only indirectly, or directly in the most tentative fashion.

Thus we have left the great and significant group of communicable diseases usually associated with the discharge from the mouth and respiratory organs of infective secretions and exudates in which the fact of aerial transmission is clear and frequent. In this group are influenza, whooping-cough, pulmonary tuberculosis, diphtheria, cerebrospinal meningitis, and the various forms of exudative infectious pneumonia. It is in this group that many complex and urgent questions of prophylaxis center. Each of these diseases presents special conditions and individual problems.

For in order to gain a just estimate of the aerial communicability of any infectious disease in which the clinical evidence is not clear and abundant, it is necessary to consider the portals of entry of the special infective microorganism and the ways in which it may leave the body; its capacity for re-

sistance to drying, sunlight, heat, cold, etc.; the condition in which it is given off, whether in fine particles or in masses more or less mingled with exudate which may materially modify its capacity for suspension in the air as dust.

We know that the tubercle bacillus may remain alive and maintain its virulence for long periods and under all conditions necessary for its complete desiccation and the grinding of the secretion which contains it into a powder so fine that it floats for a considerable time in the air. Similarly, though not to the same degree, the diphtheria bacillus, streptococcus, and staphylococcus resist drying for considerable periods.

On the other hand, the pneumococcus, the influenza and plague bacilli, and the diplococcus of cerebrospinal meningitis, like its relative the gonococcus, are all readily killed by drying.

The recent studies of Wood on the destruction of life in the pneumococcus by drying are most interesting in this connection.

But the capacity of a pathogenic microorganism to resist drying as a factor in aerial infection has become less significant since we have learned of the widespread diffusion of bacteria in the air by spraying in coughing, sneezing, etc., from the infected nose or mouth. Thus it is that the clinical evidence of communicability in the infectious respiratory diseases is not only sustained but largely extended by the direct studies upon the infective organisms themselves.

We have abundant evidence of the communicability of the air-borne infective agent in pulmonary tuberculosis and this has been generally accepted. We have quite as valid evidence of communicability in various phases of pneumonia, in influenza, in whooping-cough, and in epidemic cerebrospinal meningitis. But this has not yet become a part of the common professional belief, so that still in hospitals, asylums, and private practice, the necessary precautions against aerial communication are not enforced.

When we come to sum up the real risks from dusty air to which one is subjected indoors under the ordinary conditions of life, particularly in cities and towns, we see that they are very variable and depend upon a great many conditions.

There is more or less dust wherever man goes. It is usually most abundant and significant in densely

inhabited regions and especially indoors. Under many circumstances the dust is moderate in amount, does not contain infectious material, and so forms one of the many conditions in modern life which, though detrimental to health, are not positive and direct incitants to disease.

The protective mechanism of the body is so nicely adjusted to the usual conditions that a moderate amount of dust in the air inspired is readily and safely cast off or otherwise disposed of.

On the other hand, there are many conditions under which the dweller in modern towns finds himself, in which the air is heavily and almost continuously charged with dust, some of it undoubtedly derived from the infectious waste of diseased persons who, through spitting or unguarded sneezing or coughing, or in other ways, have contaminated the air. But whether under these unfavorable conditions one shall suffer from his exposure to these various disease incitants depends in the largest measure upon his physical condition. If the natural safeguards against infection through his respiratory organs are in good working order; if he be not predisposed through some general physical disability to the incursions of such infectious microbes as may have survived the adversities of drying, sunlight, etc., to which his particular dose of dust has been subjected—then, indeed, he may for months and years together sustain without much obvious damage the burden of dusty and even infective air.

This is in fact the good fortune of most city dwellers. But now and then, here and there, with one or another of the crowd, the adverse conditions fit together and one or another falls victim to preventable dust disease.

If now we turn to those individual precautions and public measures by which we may strive to reduce the risks of aerial infection through floating dust, it is at once evident that there are two distinct parts to the problem: *First*, the prevention of the distribution of infective material in such condition that it floats in the air or may be converted into dust; and, *second*, the practice of such methods of cleaning as shall ensure the removal or destruction of infective or other dust from inhabited places and especially from dwellings and indoor places of assembly.

First, as to the distribution of infective material. It is fortunate that, so far as we know, very few of the infective microorganisms flourish or even sur-

vive for long periods outside the bodies of men and animals. Some, indeed, such as the germs of tetanus and anthrax, in the form of spores, may remain alive for an indefinite time; but dust infection from these sources is extremely uncommon and may, for our purposes, be left out of the account. So also we need not consider here the infective agents which are not likely to incite disease of the respiratory organs, such as the typhoid and cholera organisms.

We cannot discuss, except in a general way, the exanthemata because we do not yet know just what their inciting agents are. It is probable that they are extremely minute, air-borne organisms and that infection takes place through the mucous membranes of the nose, mouth, or respiratory passages. If this be true whatever measures are efficient against the distribution of infective dust in general will be, in a measure, useful with them also, since however minute they may be they must be particulate bodies and subject to the general laws of dust transportation and subsidence. However, in the case of the most important of the exanthemata—smallpox—the protective action of vaccination has largely reduced the risks from aerial infection.

Now in this class of diseases, which we may regard as most important from our present point of view, are, as we have seen, influenza, whooping-cough, pulmonary tuberculosis, diphtheria, various types of pneumonia, various forms of nose, mouth and throat lesion associated with streptococcus and staphylococcus, and cerebrospinal meningitis. In nearly all of these diseases exudates are cast off in varying amounts which contain virulent micro-organisms.

It is clear that the golden moment for prevention is when these various infective excreta are cast out of the body. The methods for preventing the distribution of these are so well known that we need not rehearse them here. Two points, however, may be briefly touched upon.

First, the careful disposal of sputum is seldom practised to-day except in the case of pulmonary tuberculosis. Every reason for care which applies to this disease is pertinent also in nearly all of the others. Second, the wide and obvious distribution of infective stuff in a fully virulent condition by coughing and sneezing is still ignored. If, whenever it is practicable, the obvious precaution were taken to hold the handkerchief before the mouth and nose during these acts a very important advance

would be secured in the prevention of the infectious maladies which we are now considering.

For this material, if it does not find its way directly to the respiratory organs of others, soon dries in the air and is then floating dust instead of floating spray, or dries upon the surfaces to which it has settled and then is subject to the various physical conditions under which it is ground into a powder.

The filthy and wholly unnecessary habit of spitting on the floors in public places and in many dwellings is still widely practised. The few instances in which legal measures have been resorted to to suppress this nuisance and menace to the public health have already led to a noteworthy sentiment against promiscuous spitting.

But in order to secure important results, offenders must be ceaselessly and relentlessly pursued. An occasional arrest at long intervals is of little value and whatever official shall undertake to do his duty in the matter will have to realize that the fish who come to his net are as likely as not to be quite exalted political personages, as well as others in fashionable attire.

But education in cleanliness is a slow and discouraging undertaking and involves a far more radical reform in the sense of decency than the law alone can ever secure. Moreover, under the most favorable conditions there will always be a considerable dispersal of infectious material in closely crowded communities.

Thus we are led at last to a consideration of those methods of cleaning and disposal of dust which our knowledge of the infectious diseases makes imperative and which our studies of dust have made entirely practicable.

In all living rooms the conditions are favorable for a continuous accumulation of dust. If there be an adequate ventilating system the so-called fresh air in our cities usually enters more or less charged with soot and other forms of dust. This in part settles upon the horizontal surfaces before the contaminated air leaves the room through its proper exits. The ordinary gas burners furnish an enormous number of minute smoke particles invisible to the eye. Shoes and garments worn upon the streets and in places of public assembly bring into dwellings large quantities of material of the most objectionable and often filthy or infectious origin, most of which presently takes its place with the rest of the floating and falling dust. Add to this

the sprayed secretion in coughing and sneezing, the wear and disintegration dust from persons, garments, furniture, carpets, hangings, etc., and one has framed but an incomplete inventory of the aerial flotsam and jetsam of an inhabited room.

While dust in a condition to do harm is always floating in the air it is important to remember that in the long run dust is always falling. It is true that when the particles are small and light they may be temporarily swept upward or borne along on air currents so that the effect of gravitation seems to be temporarily suspended, but the steady pull is downward and they always come to rest on the lowest available surfaces. Thus it is that in enclosed places the air always tends to free itself from dust.

If some time in the twenty-four hours the room can be shut up so that the air is fairly still the dust will practically all settle to the horizontal surfaces. If now we can remove it from these without stirring it into the air again we may, once in the day at least, start with relatively dust-free and germ-free air.

If the rooms are uncarpeted it is easy, by the use of moistened sawdust, to sweep them with comparatively little stirring of the dust. Then if two or three hours be allowed to pass with the rooms closed or still, whatever dust may have been stirred in sweeping will have again settled and by the use of moistened mops and moist or dry cloths it may all be removed.

But as a rule this is not done. Dry sweeping is largely practised and this is, under all circumstances, an abomination. Then usually the so-called dusting is done before the proper time has been permitted for the dust to settle. Then, further, the most silly performance conceivable is indulged in, namely, instead of removing the dust, which has settled, in a safe and easily managed condition, it is set afloat again by dry dusting. Nothing is accomplished by this except to get the dust off from the shiny surfaces for a short time and to get it in a condition to do the greatest possible harm through its inhalation by the unfortunate victims of that most fatuous of housekeeping fetishes, the feather duster.

There appears to be no good reason why the simple procedures above recommended should not always be followed in those places of public assembly in which, at present, the risks of dust infection are most constant and most threatening. Such places are theatres, churches, clubs, schools, court

rooms, factories, stores, office buildings, hotels, railway and trolley cars, hospitals, asylums, and the numerous rooms in which artisans are crowded together engaged in occupations involving the development of various forms of dust.

If all such places in which it is practicable were properly swept *at night* after their occupants had left them, and then closed until morning, when, before the people returned, with moist mops and dust cloths the floors and all horizontal surfaces were intelligently cleaned, with the purpose of removing and not simply stirring up the dust, there would be an immediate and far-reaching reduction of the dangers from infectious and other floating matter in the air.

Of course in private dwellings the methods of keeping the air free from dust which commend themselves in public places are not wholly applicable. Sweeping cannot be done at night, and the elaborate furnishings which are in vogue make special procedures necessary. The scope of this paper does not permit me to enter upon these in detail. But here as well as in all carpeted places the use of moistened shreds of paper thrown upon the carpets and carried forward with the broom will be found most effective in gathering and holding the dust.

The vacuum process in cleaning, if this be properly administered, affords a valuable means of getting safely rid of a considerable amount of dust which has gathered upon carpets, rugs, and other surfaces. But the plants for this purpose have not yet been widely installed, and in daily routine it is wisely combined with other and supplementary methods of dust removal.

The carpeting of places of public assembly involves the presence of filthy and dangerous dust in the air, and until proper notions of intelligent cleanliness shall prevail the frequenters of such carpeted places will pay the penalty of their indulgence by attacks of disease of the respiratory organs which might readily be avoided.

The chief error in the present situation is that these homely but necessary offices of daily cleaning are not intelligently directed and supervised, but are placed in the hands of ignorant persons, entirely willing and often furiously devoted to their tasks, but who are seldom advised as to the necessity for the removal, not the stirring up, of dust. The more devoted they are the more often do they appear with the feather duster to set astir the dust which for

the moment may have settled to some inoffensive place upon the furniture or floor. The result is that in some of the most carefully and expensively administered places—the great city clubs, for example—the members are perpetually served with the most obviously dust-laden air.

It is a curious fact that in a large proportion of the various places of public assembly the only systematic and effective measure of removal of the often infectious dust is by the mucous membranes of the nose, mouth and upper air passages of the persons who frequent them. One inclined to figures may readily estimate the efficiency of this method of dusting a theatre, for example, if he considers that at each breath the average person takes into his respiratory organs about thirty cubic inches of air. This he immediately discharges, wholly purged of its dust and microorganisms which he retains and must presently dispose of as best he can. But the air of the theatre is free of them.

The trouble is that while some of us are mighty particular about the cleanliness of our clothing, of our food and drink and the utensils in which these are served, and the exterior of our persons, we make no protest as we bear away upon the delicate membranes of our respiratory organs, from places of public concourse, the pulverized excretions of the filthy and diseased. Fancy our reproachful protest should the breakfast of to-day be served upon utensils marked with the remnants of yesterday's repast! But in a considerable proportion of cases in theatres and court rooms, in offices and public conveyances, in factories and stores, the floating dust of to-day is pregnant with the spit of yesterday.

The tendency of the hour in cities is to cram people into box-like apartments in great, many-storied buildings. And while, in administering to the welfare of the tenants of these vast caravansaries in lighting, heating, plumbing, ventilation, etc., the most refined requirements of the day are not overlooked, it still remains a fact that against the growing menace of floating matter in the air there is seldom any safeguard. Whatever degree of intelligence the janitor and his satellites may bring to their tasks the tenant may thank his good fortune for. But he usually clears his air of dust by his own mucous membranes, and he is usually the victim of chronic nasal or pharyngeal catarrh.

Would it not be well to release our delicate and valuable breathing apparatus from the burden of the

disposal of this contribution from all manner of men, animals, and things, and give it a chance to settle at least once a day and then, once for all, get rid of it?

Of course this does involve the higher education of the housekeeper and a certain degree of trained intelligence in the executives—and the total abolition of the feather duster.

Of course in time the steady breathing of dust-laden air does free it from its floating matter, but it is an expensive method of housekeeping and the final account is made up in the disease and death records of the Health Department.

It seems a little difficult for laymen—and for many physicians, too, for that matter—to realize that there is no mystery about the way in which the agents which incite infectious disease are conveyed from one to another. The tendency seems to be to look for some intangible miasm, some vague, half personified agency which moves about with sinister intent, and to insist that the only way to remove the risks of aerial infection is to use some more or less choking or bad smelling disinfectant, or to saturate the air with a highly aromatic vapor which may mask, but does not destroy, bad odor due to faulty sanitation.

The fact, however, is that a large part of the so-called disinfection might be replaced, to advantage, with proper methods of sweeping, dusting, and mopping, if these were not so simple and commonplace and all could and would realize the significance of floating dust in the conveyance of infectious agents.

But while the initiative and the moral support in such measures for safeguarding the public health must come from the medical profession, I should still have hesitated to direct the attention of the Practitioners' Society at such length to a subject so elementary and so time-worn, were it not that a specific and urgent responsibility appears to rest upon all physicians in the protection of patients in the hospitals to which they minister, against the dangers of infectious and other dust.

The large number of cases of secondary infection acquired in hospitals, and particularly in children's hospitals and asylums, is fairly appalling, especially in the winter months, and, while many factors are doubtless concerned in maintaining this sinister condition, there can, I think, be little doubt that aerial infection through dust-borne microorganisms plays a large and significant part.

The general aspect of most modern hospitals is one of extreme cleanliness, and certainly in most instances there is no lack of attention and willing service in the daily routine of cleaning. But, in fact, except in the case of tuberculous patients who may be admitted to general hospitals, there is no systematic attention paid to the directly infectious zone which surrounds each patient suffering from an infective disease of the respiratory organs, formed by the dispersal of secretions through sneezing and coughing.

Furthermore, the processes of daily cleaning are almost never specifically directed to the absolute removal of the finer dust.

I do not think that those concerned with the so-called dusting of hospital wards realize how large a proportion of the horizontal surface on which dust settles is made up by the floors and beds. A few square feet at most of tables, chairs, and window seats are attended to, but the larger part remains, to cast its accumulation into the air by the stirring of the beds and the tread of attendants and visitors upon the floors.

I need not detain you with a detailed description of the current methods of ward and corridor cleaning in hospitals. But in fact the one procedure by which the most satisfactory removal of dust may be secured is not practised. I refer to the use of moist mops on the floors, and moist dust cloths on the solid furniture, and the careful removal and shaking out-of-doors of the night counterpanes, *all in the early morning before the stir of the day begins.*

Sweeping, if this be necessary, should be done before the quiet of the night is established. Then during the relative stillness of the night hours practically all dust and all floating infectious stuff will fall to the beds and floors and horizontal surface of the furniture, whence it may readily be removed. Thus once in the twenty-four hours a hospital may be largely freed from its irritating and infectious air-borne contaminations.

In speaking of the dusting of floors by a mop, I do not mean the usual mopping with the abundant use of water which leaves the floor more or less wet. I mean the use of a large mop, thoroughly wrung out so as to be only moist, not dripping, which is to be passed rapidly over the floor, touching but not lingering upon each part. In this way, as fast as the operator may walk, the whole floor surface is covered and a large area may be effectively cleaned of

its dust before it is necessary to rinse and wring out the mop anew.

Of course the initiation of this procedure involves some slight change in the hospital cleaning routine commonly in vogue, which is largely directed toward the removal of visible dirt. But after all, the only essential change is in the moist dust removal, as indicated, in the early morning before it has been in any way disturbed.

Of course it will be said that this procedure is impracticable, that it is expensive, that it is useless, that it is based on finical, theoretical postulates, and savors of the laboratory. However, it has been the writer's good fortune to secure the co-operation of the administration in two large hospitals and in one large educational institution, in having the wards and public assembly rooms properly swept at night, while in the early morning before the wards were astir or the assembly rooms occupied, the settled dust was all removed in the manner indicated and safely sent to sea by the sewer.

In each instance, after the reluctance of the housekeeper to listen to the vagaries of sanitation had been overcome, the results have been most striking and satisfactory; nor has the expense of the change of routine been noteworthy.

In fact, it is much cheaper to get rid of the dust day by day than to keep it stirring, trusting to the chances of the hour that some of it will blow out of the window, or relying upon the friendly offices of the mucous membranes alike of the well and the sick.

In most modern hospitals much more money is wasted in overheating the wards and in blowing prodigious volumes of air out of opened windows in futile directions, that would be required to remove the dust in the most effective fashion. But this does require conviction, persistence, and unceasing supervision.

The tendency of modern medical practice to look at the sick man rather as a halting or disabled machine than as a diagnostic abstract to be dosed, as was so long the vogue, should, I think, lead the practitioner at least to a sympathetic attitude toward the efforts of modern hygiene to maintain an intelligent cleanliness of the air.

In all the delicate mechanism by which the healthy individual may, at need, protect himself against the ill effects of dust and infectious microorganisms, inspired in the air he breathes, the sick man is usually

sorely handicapped. To put upon him the necessity of overwork by either his mucous membranes, his lymph nodes, or his phagocytes, or all of these together, is surely not more commendable than it would be to stuff with indigestibles a digestive apparatus of supreme importance in the emergency of illness, or in need of rest.

In view of the increase in respiratory diseases in cities, it is interesting to note that while water supplies are now at last usually subject to the most careful supervision on the part of municipal health authorities, while in well regulated towns food supplies are looked after with more or less intelligence and zeal, one of the three great necessities of human life, the air, which we are ceaselessly taking into the most delicate of our organs, is left almost wholly unguarded either by public supervision or private attention against wilful or careless pollution by the filthy, the vulgar, and the diseased.

While we are concerned in this paper especially with the accumulations of dust indoors where if proper methods of cleaning be not practised it is stirred again and again into the air we breathe, there are certain conditions out-of-doors in New York which deserve at least a brief mention.

An examination of the sanitary conditions in the subway is now being made by Dr. Soper for the Rapid Transit Commission, the result of which we may wisely await. Already many most interesting and significant facts have been brought to light. It is to be hoped that the Commission will realize the far-reaching importance of this research and see that it is carried to completion, so that we may definitely know in the future what to do and what not to do in the construction and administration of these important avenues of transportation.

But the stairways and platforms of the elevated railway have now, for many years, been swept with gross disregard of the welfare of its patrons, who are forced to breathe clouds of the most filthy dust when the attendants are sweeping. No public protest seems to be of avail with those responsible for this abomination. When the porters of the system feel like sprinkling the floor and stairs before sweeping, they do so; when they see fit, they omit it. Individual protest to the company meets with courteous response and the dry sweeping goes on just the same. The Department of Health has long been aware of this nuisance and danger. It has the power to stop it, but does not do so.

There was a time in New York when the street sweepers were required to sprinkle the pavement in advance of their brooms. But of late we are forced to lend to the Street Cleaning Department the good offices of our respiratory mucous membranes already overtaxed by the requirements of the theatre man, the storekeeper, and the trolley car folks, by the traditions of domestic service, and by the house committees of our clubs.

If, in New York, the beneficent law against the smoke pollution of the air were enforced; if the streets were properly cleaned; if the great passenger transport systems were placed under competent sanitary supervision; if the Health Department were persistently alert in enforcing the ordinance of the sanitary code which relates to spitting in public places; and if we could get sweeping and dusting indoors intelligently done, we should have in this city, in my opinion, a fair outlook towards a great advance in the reduction of diseases of the respiratory organs. These are indeed large and difficult "ifs" and they do not appeal as strongly to the public as does the search for a new "serum," but the demand which they imply seems not unreasonable.

The condition which we face is in short this: The infectious diseases of the respiratory organs are steadily increasing as people are more and more huddled together in offices, dwellings, traveling conveyances, and places of public assemblage. A large part of these diseases are directly traceable to infectious material cast off, in spitting, coughing, and sneezing, from the mucous membranes of those suffering from various grades of local disease, and floating for longer or shorter periods in the air as dust or invisible spray.

Promiscuous spitting can and should be stopped. Coughing and sneezing cannot be prevented, but in most instances their harmfulness can be controlled by the use of the handkerchief, or, in case of need, by such use of the hand as shall answer the dictates of primitive decency. This is a matter of public education. But when all is said and done in these directions there remain the humble offices of cleaning which can only be effective when conducted with trained intelligence.

The private citizen can stay away from the theatre and the opera, and this, if largely practised, would prove a powerful educational influence upon some of the worst offenders against the salubrity of the air. He may or may not be able to control his own

office and workshop, but he can make the attempt. As for his home, he is lord of the castle—sometimes—and he may have clean air in abundance at the cost of a little vigilance and common sense; but he may, if he choose, and he usually does, contaminate his air with the floating refuse of the street and the wear and tear of his person and belongings, and breathe it so—*chacun à son goût*.

But as regards public conveyances and places of public assemblage, I see no reason why health authorities should not as properly prescribe regulations for cleaning and the removal of dust, and compel their practice, as they compel the sanitary disposal of sewage, secure pure drinking water, and condemn noxious articles of food.

**CHORIONEPITHELIOMATOUS PROLIFERA-
TIONS IN TERATOMATA,
ESPECIALLY IN THOSE OF THE TESTICLE; WITH THREE
NEW CASES.***

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1. INTRODUCTION.

I. GENERAL REMARKS.

In recent years a peculiar form of tumor, known as chorionepithelioma, has been noted in connection with pregnancy. Since attention has been directed to this growth the reported cases have rapidly multiplied, so that they no longer should be regarded as a pathologic curiosity, but rather as a dangerous neoplasm with which the clinician may be confronted at any time, and which may convert, without warning, an apparently normal pregnancy into a rapidly fatal illness.

Hardly had these tumors become generally known, when renewed and added interest was aroused by the discovery of similar tumors in teratomata of the testicle. The resemblance of these two varieties of growths, though occurring under such diametrically opposite conditions, was too striking long to escape notice. It has been the effort of numerous workers to prove the kinship of chorionepitheliomata connected with pregnancy with those of teratomata.

In order to make clear the fundamental facts known about chorionepitheliomata it will be necessary to define these neoplasms, and the terms employed in describing them, although a more detailed account will be given later.

The chorionic villus, in normal pregnancy, is covered by a double layer of epithelium, of which the

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basal layer, variously known as the Langhans' cells (from their discoverer) or *Zellschicht* consists of clear polyhedral, mononuclear cells, containing glycogen. The covering layer is composed of larger protoplasmic complexes, giving on section the impression of polynuclear darkly staining sheets. They are known as syncytium. In the uterine musculature certain transitional types, called for convenience sake chorionic wandering cells, are found. In staining properties and general appearance they resemble the syncytium, but in size they are intermediate between the syncytial type and the *zellschicht*.

These three types of cells, all of fetal derivation, are regularly met with in the normal villous covering of the embryo and collectively may be termed the trophoblast. In the female, malignant tumors, directly continuous with the chorionic villi (often these villi are diseased, hydatid mole) and composed of the three types of cells just described, are known as chorionepitheliomata of pregnancy (Marchand). This chorionepitheliomatous or trophoblastic tissue was considered a distinct variety of tissue, peculiar to pregnancy, just as special as epidermis for instance. When, however, morphologically similar growths were found, quite independent of pregnancy, (in the ovaries of virgins and still more frequently in the testicle) this conception received a rude shock. The teachings of Marchand appeared discredited and, in fact, the so-called English Chorionepithelioma Commission was induced to hand in an opinion declaring that the tumors in question should be classed as decidual sarcomata, as they had been regarded until 1895.

When the derivation of chorionepitheliomatous tumors of pregnancy, however, is compared with that of morphologically similar tumors found in the testicle or elsewhere, a similar derivation can be demonstrated. For the chorionepitheliomata of the testicle were, in most instances, an integral part of a teratoma, and the genesis of teratomata, as will be shown later on, is comparable in many ways with the processes of gestation.

With these preliminary considerations disposed of, the reader is ready to study the series of cases, and the theoretical deductions based on them which led Marchand and others to the interpretations now commonly held.

CHORIONEPITHELIOMA IN THE FEMALE.

Early Cases and Their Interpretation as Sarcomata.

—R. Maier¹ and Sanger² are credited with the recognition of chorionepithelioma as a new and distinct variety of tumor. Maier regarded his two cases as a disease of the decidua of pregnancy. Sanger and Chiari-Pfeiffer³ independently described their cases as malignant metastasising sarcomata of the decidua, without according any share to either epithelial or glandular constituents. Before these authors had called attention to this new field, such tumors probably were classified among the carcinomata or sarcomata of the uterus, as are the three cases of Chiari⁴ published in 1877.

Marchand⁵ was the first to champion the epithelial nature of chorionepithelioma (unless we except the cases just mentioned, in which the tumors were mistakenly grouped with ordinary carcinoma of the uterus). In his first article he considered the syncytium of maternal origin, and was inclined to regard the simultaneous participation of the fetal ectoderm (the Langhans' cells), with the maternal syncytium as a symbiotic growth. Since then⁶ he has leaned more strongly toward the genetic unity of these forms, at first according the possibility of both a maternal as well as a fetal syncytium, and in a still later article,⁷ in 1903, going so far as to state that Langhans' cells and syncytium are not only of similar origin but are interchangeably capable of assuming either the one form or the other in response to different functional requirements. R. Spuler⁸ had arrived at similar conclusions as to the genetic identity of the two layers in a rather unconvincing study based on the morphologic similarity and transition forms seen in the covering of two hydatid moles; this work has received the support of C. Ruge.⁹

The Relation of Chorionepithelioma and Hydatid Mole.—An intimate but not clearly understood connection between chorionepithelioma, hydatid mole, pregnancy and abortion will be noted in the cases to be described, but at the outset this connection was not correctly interpreted. Especially intimate was the relation to hydatid. According to the statistics of Teacher,¹⁰ based on 188 cases of chorionepithelioma, 74, which is 39 per cent., were known to have had a preceding hydatid. In how many instances the mole was discharged unnoticed can never be determined.

The intimate relationship to hydatid naturally led to renewed study of this growth, and to a change of previously accepted views. Virchow's¹¹ dictum, which classified hydatids as a myxoma of the placenta in which the stroma played the active rôle, was finally discarded, and again it is Marchand's views¹² which have been fully accepted. Accordingly, the stroma has been relegated to a secondary place, the swollen, translucent appearance being ascribed to hydropic changes. The active rôle is assumed solely by the chorionepithelium, both layers proliferating with undetermined and variable intensity. As observations increased in number, hydatid moles were found, in rare instances, to develop malignant and destructive qualities causing death from metastases (Salowij-Krzyskowski¹³); others produced metastases but showed no really malignant properties, the patients recovering without radical operative measures (cases of Pick,¹⁴ Schlagenhauser, Case 1¹⁵).

Morphologic Criteria of Malignancy.—In view of the great practical importance of determining the malignant or benign properties of such growths as soon as observed, numerous authors have searched for some morphologic criteria on which definite prognostic value could be based. Neumann¹⁶ regarded the large syncytial cells, occasionally seen in the stroma or just beneath the epithelium, as signs of malignancy, but Pick, Ruge and others have discredited his work. Various other characteristics, such as the increased size and richness in chromatin of the nuclei of the syncytium (Gottschalk¹⁷), or the presence of numerous chorionic wandering cells (Ruge¹⁸), have not stood the test of time, for similar pictures were shown to exist in normal pregnancy (Marchand, Pels-Leusden¹⁹). All recent writers agree that the morphologic pictures of malignant and non-malignant chorionepithelioma are identical, and that the subsequent course alone can determine the outcome.

This question has again been reopened by von Velits,²⁰ who not only claims to be able to distinguish spontaneous regression by the microscope, but also sees regressive changes macroscopically! The diminished vitality of the Langhans' cells is supposed to show itself by the absence or lessened number of mitoses; the presence of chorionic wandering cells is taken to be an addi-

tional indication of degenerative changes. Hörmann²¹ discusses these assumptions of von Velits fully, and convinces the reader that these, like the previously mentioned findings of Neumann and Gottschalk, deserve to be discarded.

In destructive hydatids within the uterus, no difficulty was experienced in showing direct continuity between the chorionectoderm and the advancing tumor cells (Neumann, Gebhard²²). In metastatic growths, Pick¹⁴ first demonstrated this continuity in a vaginal metastasis of an otherwise benign hydatid; Apfelstedt and Aschoff²³ in a true chorioneplithelioma. Veit²⁴ subsequently published his views on villous emboli in pregnancy, which promised to complete the link in the chain of evidence prepared by the findings of Pick, Apfelstedt and Aschoff. Recently, however, Schmorl²⁵ has cast a doubt on Veit's conclusions, by observing that only after long and protracted labor, in which much manual trauma has been sustained, do entire villi enter the circulation. In hydatids or eclampsia, if emboli do occur, cells and not villi are transported. This again emphasizes the fact that the cells and not the stroma are the active agents.

Ectopic Chorioneplithelioma.—Schmorl²⁶ reported a case in which, after the complete expulsion of an hydatid, chorioneplitheliomatous metastases developed throughout the body, the uterus and tubes, however, showing no changes. These cases, classified under the name of ectopic chorioneplithelioma, have multiplied, until recently twenty-one were collected by Findley.²⁷ In many, a previously occurring hydatid, which was either operatively removed or spontaneously expelled, had been noted; in a few a supposedly normal pregnancy or abortion, without observed mole formation, was regarded as the etiologic factor. This would oblige us to assume that the normal fetal placental tissues can be the precursors of such malignant growths. Against such a doctrine Schmorl's work and the so frequently observed preceding placental degeneration bear strong weight. Instances of only partial hydatid change, insufficient to destroy the fetus, or twin pregnancy in which one of the children is normal, the other reduced to an hydatid rest, have been reported (see Marchand). The chorionic changes may be so small in extent as

to escape notice, and therefore we should accept only with great reserve cases apparently arising from a completely normal placenta, unless this structure has been subjected to careful examination. As the malignant course develops only subsequently, such complete observation is usually not made.

Increase of the Lutein in Hydatid and Chorionepithelioma.—The discovery of an increase in the lutein of the ovary during the course of hydatid or chorionepitheliomatous growths, led to the conclusion that there might be a causative relationship. Some authors went so far as to consider this hyperplasia, which was found in the form of lutein cysts, as a direct stimulus to the chorion ectoderm, which then developed increased proliferative activity and malignant qualities. A contrary view is expressed by Seitz,²⁸ who, examining the ovaries of thirty-six women between the second and tenth months of gestation, found lutein cysts and a varying increase of lutein, in addition to that of the corpus luteum, in all but one case. Patellani²⁹ still more recently reopens the discussion by showing that in a large percentage of chorionepitheliomata the ovaries, in consequence of the lutein hyperplasia, assume a characteristic sausage shape, and undergo marked increase in size, sufficient to prove of diagnostic value. This phase of the question must as yet be considered unsettled.

The specific character of the growth is well illustrated by the decidual changes observed in chorionepithelioma away from the placental site, analogous to those seen in ectopic gestation (Schmorl, Risel,³⁰ Holzapfel³¹).

The Causes of Chorionepithelioma.—The opinions and theories advanced to account for the causation of chorionepithelioma are almost as varied and numerous as those entertained about the etiology of tumors in general, and may prove a valuable argument against a parasitic origin of carcinoma (Ribbert,³² Pick³³). Marchand believes nutritional changes and chemotactic influences at fault, and that the earlier in the course of a pregnancy such abnormal growth begins the more malignant the subsequent course will prove, though originally³⁴ he inclined to Ribbert's opinion, that a loosening of cells from the general complex afforded the starting point. Veit³⁵ still regards the maternal uterine tissues as the starting point of the growth, which, according to this author, begins before pregnancy. A somewhat modified

view is held by Pfannenstiel,³⁶ who turns to the endothelium of the maternal decidua for the origin of the tumor.

Death of the fetus, softening of the uterine wall, etc., have all been assigned as possible causes. Schmorl declares, however—and his work is based on weighty arguments—that most likely in every instance a diseased placenta, probably hydatid, has preceded. Of the 158 cases he examined for emboli he found proliferation of the cell emboli in one eclamptic only, who died ten days postpartum. As he has seen similar syncytial changes in inflammations, he is inclined to disregard this case. Of the other cases the emboli were numerous in three cases of hydatid, and in three cases of abortion in which neither uterus nor ovaries had been subjected to examination. In some of these cases the cells appeared to be undergoing active proliferation, giving the picture of an early metastasis of an atypical chorionepithelioma. These observations, however, bring us no nearer the real primary cause of chorionepithelioma than before.

Experimental Work.—Little experimental work has been performed. The placental emboli of Lengemann,³⁷ Lubarsch³⁸ and Maximow³⁹ have shed no light on the subject. Aichel,⁴⁰ in dogs, obtained structures similar to hydatids by subjecting parts of the placenta to pressure. Such resemblance appears incomplete, and no observations as to the final outcome of these artificially produced hydatids are on record. Vassmer⁴¹ implanted portions of a chorionepithelioma into the peritoneum of rabbits without result. Birch-Hirschfeld and Gartner⁴² were equally unsuccessful with placental tissue introduced into the jugulars of two goats.

For further and fuller accounts of chorionepithelioma of pregnancy the reader is referred to Marchand, Risel, Teacher and Findley.

CHORIONEPITHELIOMA IN TERATOMATA OF THE FEMALE. RECORDED CASES.

Thus far I have dealt solely with those chorionectodermic tumors which showed a distinct relation to pregnancy. Similar tumors have been observed, however, in patients in whom pregnancy could be positively excluded, either through youth or sex. As chorionepitheliomata of the testicle will be discussed in detail later on, only such as have been observed in females

will be treated of here. If the case of so-called hydatid mole in a virgin of 12½ years, passed at her fourth menstruation (Bock⁴⁴), were to be regarded as authentic, it would furnish an example of an hydatid on a congenital basis, but as neither the macroscopic or microscopic description is complete or convincing, this case must be discarded entirely.

Lubarsch⁴⁵ describes a large retroperitoneal tumor in a girl of 13. The case is incomplete, as the material was obtained at operation and the patient returned to her distant home, no autopsy being permitted. The tumor showed all the characteristics of a chorionepithelioma and we must ascribe its origin to a teratoma of the ovary, unless we accept Risel's view that in spite of the tender years of the patient a pregnancy can not be excluded. Lubarsch himself was inclined to consider the resemblance a result of metaplasia forming a *Doppelgänger* of this type of tumor.

Pick's⁴⁶ case of Hedwig R., in which chorionepithelioma was found in a teratoma of the ovary of a child 9 years of age, can not be subjected to a similar imputation. Here the chorionepithelium was in spots in direct continuity with neuroepithelium. In some regions the growth assumed more nearly the typical form of Marchand. Other ectodermal, in addition to mesodermal and endodermal constituents, were also found.

Michel⁴⁶ reports an analogous case of "carcinoma" of the ovary, which he offers in support of the non-specific value of such chorionepitheliomatous tumors, but far from agreeing with him, I consider that Pick⁴⁷ proves that this tumor is of similar origin as his own case of Hedwig R.⁴⁸

In order to grasp fully the significance of chorionepithelioma in teratomata and to account for their occurrence in connection with these growths, it will be necessary to take a rapid survey of our present views on teratomatous tumors.

THEORETICAL CONSIDERATIONS; AND EMBRYOLOGIC SIGNIFICANCE OF TERATOMATA.

Wilms,⁴⁹ in his earlier works believed that true teratomata could be found in the ovary and testicle alone, because he derived them from a *Geschlechteszelle* and considered dermoids elsewhere in the body as genetically distinct. Bonnet⁴⁹ regarded the frequent occurrence of teratomata in the male and female generative glands

as due to the comparatively large size of the urogenital anlage in the fetus, which would account for the more frequent location of independent blastomeres in this region, but does not entirely preclude their occurrence elsewhere. Traina⁵⁰ ascribes it to the exceptionally favorable nutritional conditions which he experimentally determined in the ovary.

Numerous observations of teratomata at other sites, as in the abdomen (where some skeptic might still suspect a hidden connection with the generative glands), and in the thorax and head, are, however, on record. Ekehorn⁵¹ has collected no less than thirty-one cases of mediastinal dermoids. Many of these showed the three fetal layers once demanded as the sign manual of true teratomatous growths. A case of teratoma of the mediastinum with chorionepithelioma has been recorded by Ritchie and will be considered more fully later on. Dermoids and teratomata of the brain, bladder; coccygeal region and liver are also known. We can no longer accept Wilms' first view, which placed the embryos or teratomata of the ovary or testis in a distinct and separate class. The Marchand-Bonnet theory,⁵² which accords a genetic equality to all composite dermoids, embryos and parasites, is now agreed to by nearly all. Derivation from a blastomere or impregnated pole cell, which at an early stage has been cast out of the complex, will account for the three embryonal layers found in these growths. The earlier the liberation takes place, the more fetus-like and complex will be the resulting growth. Bonnet claims that an almost unbroken line can be traced from the *acardiacus amorphus*, on the one hand, to teratomata, in which the atypical proliferation has reached so high a degree that apparently simple tumors result. That not all layers need reach an equal degree of development is proved by the solitary tooth found isolated in ovarian stroma (Saxer⁵³), and interpreted as an unequally developed teratoma; or the *struma colloides ovarii* (Pick⁵⁴, Walthard⁵⁵), in which one tissue had developed at the expense of the others, either hiding the other constituents, or completely replacing them. Whether we should regard the blastomere or pole cell as the origin of teratomata has never been decided. This point will be touched on later.

The cases of chorionepithelioma occurring in teratomata of the testis are widely scattered in monographic

literature, although Risel has collected them to the year 1903. I will therefore briefly present all the available cases, but limit myself to their salient features.

2. CHORIONEPITHELIOMATA OF THE TESTICLE.

Nineteen cases of chorionepitheliomata of the testicle have been recorded. Some of these were at first regarded as sarcomata, and have only subsequently been reclassified under this new head. A detailed description of the clinical and morphologic characteristics of these tumors will be found in succeeding sections; in this section the main features of the cases, and such deviations as will prove of special importance in the further discussion, or of special historical interest, alone will be considered.

In all the following cases (with the possible exception of Boestrom's) a primary testicle tumor was noted; and, in all which did not escape subsequent observation, death from metastases occurred within a comparatively short time. Microscopically, the chorionepitheliomatous portions will be found classified as either typical or atypical (see Section V), unless described in detail.

Three French reports, those of Malassez and Monod,⁵⁶ Carnot and Marie,⁵⁷ and Ch. Dopter,⁵⁸ may be grouped together. These authors regarded their cases as sarcoma *angioplastique*, in which tumors, the large syncytial complexes, composed of darkly staining, vacuolated protoplasmic masses with many nuclei, attracted their chief interest. Because of the intimate relationship of the syncytia to the smaller blood vessels, and to extravascular collections of red blood cells, these authors ascribed vaso-formative properties, such as Ranvier had noted in the *taches laiteuses* of the rabbit's omentum, to the syncytium. Malassez recorded only the giant cell structures, but the others also describe clear, polygonal, uninuclear cells in carcinomatous, or alveolar sarcomatous arrangement. None of them either found or looked for teratomatous constituents.

Wlassow⁵⁹ added four cases to the literature. The data are incomplete. The major portion of each tumor was composed of clear, polygonal, glycogenic cells (of the Langhans type), arranged as in scirrhus or medullary carcinoma, or alveolar sarcoma. Syncytium was well represented in all. The metastases in Case 1 were typical. Noteworthy is the fact that the syncytium in

the splenic metastasis showed a ciliary margin. Cases 3 and 4 contained teratoid portions, in the primary tumors, represented by ectodermal and mesodermal structures (cysts lined with various epithelia, embryonal muscle, cartilage, etc.). Because no entoderm could be found, Wlassow refused to accord an embryonal derivation to these tumors, and though he recognized their resemblance to the chorionepitheliomata of the female he, likewise, disregarded this feature. According to Wlassow the syncytium has a vaso-destructive, not a vaso-formative, function. The classification he adopted was that of a carcinoma *sui generis*, because an actively proliferating mesodermal anlage acted as the connective tissue of the growth. He chose the name of *epithelioma syncyomatodes testiculi*.

Schlagenhauser⁶⁰ gave the first clear expression of the views now generally entertained. He claimed that the tumors in question are developed from teratoma, and are genetically equivalent to the chorionepitheliomata of the female. Going one step further, he assumed that rudimentary fetal membranes, in the teratoma, furnished the actual starting point for the tumor, and as evidence of the probability of a direct chorionic genesis, he cited cases of hydatid-like intravascular growths, discussion of which will be taken up later. The case he reported showed no distinctive features.

Schmorl⁶¹ published two cases. The first was described in greater detail by Steinert;⁶² the second was typical, showing both teratomatous and chorionepitheliomatous structures (the latter very limited in extent).

Boestrom's⁶³ case is noteworthy in that a small brain tumor, operatively removed, proved to be typical chorionepithelioma. Metastases in other parts of the body were of similar constitution, while the testicles were normal (?).

The case published by Steinert⁶² is of great theoretical importance, because not only were teratomatous (organoid) structures and chorionepitheliomatous tissues found in the testicle, but also in the metastases present in the retroperitoneal and mediastinal glands, and in the liver.

Steinert proposed three hypotheses to account for the teratomatous metastases: 1, Multiple blastomeres primarily deposited in the organs during early fetal life; 2, numerous blastomeres, in the primary tumor, which

later were transported to other regions; 3, undifferentiated cells transported from the primary growth, at so early a stage of their development that they were still capable of producing all three fetal layers. Of these hypotheses the last is most plausible. The first presupposes multiple anlagen with a synchronous development of malignancy at their widely scattered sites; the second requires the assumption of quiescent blastomeres, hidden amid the tumor cells, while the third most nearly approaches our preconceived and more generally accepted ideas of tumor development (Ribbert). In the metastases, the chorionectoderm, in distinction to the other tissues of the teratoma, alone evinces "malignant" properties.

Steinhaus⁶⁴ case deserves no special notice. Teratomatous and chorionepitheliomatous formations both were in evidence.

Risel⁶⁵ (p. 145) reported two cases, which show important findings.

The first, in addition to the usual teratomatous and chorionepitheliomatous portions, contains ringlike forms resembling medullary canals of neuroepithelium, which, in one spot, are directly connected and continuous with syncytial masses, and where lining cysts, show complete transitions from ciliated to cubical, or even epidermoid epithelium.

The chief conclusions drawn from this case by Risel are that two forms of embryonal epithelium are here encountered in active proliferation—the chorion epithelium, with all its characteristics, and neuroepithelium, which shows a tendency to form analogues of a medullary tube; secondly, that the transition of cells of chorionepitheliomatous appearance with other epithelial cells, amid other tissues, bears witness to their genetic unity and speaks against their derivation from a special anlage of fetal membranes. He regards them as a special form of the fetal ectoderm, which in this instance has also produced actively proliferating neuroepithelium.

These conclusions agree with those entertained by L. Pick and appear to be fully warranted. Pick has most convincingly demonstrated direct continuity between neuroepithelium and chorionepitheliomatous cells, in his case of ovarian teratoma.

The second case of Risel (p. 151) is incomplete be-

cause the primary tumor is lacking. The metastases, with the exception of the prevertebral are typical. In the prevertebral tumor are portions composed of smaller, darker, uninuclear cells, often in ductlike or alveolar arrangement and without the accompanying fibrin and necroses, typical of chorionepithelioma.

According to Risel these areas are derived either from a proliferation of the epithelium of the seminiferous tubules, of the primary growth; or, as is more probable, from some epithelial tissues contained in the primary testicle teratoma (which was not available for examination).

Emanuel⁶⁵ reported a case whose distinctive features resembled those more fully discussed in connection with the case of Askanazy.⁶⁶ The metastases were typical. The primary growth contained teratomatous formations and chorionepitheliomatous tissue which, in some parts, imitated carcinomatous, in others, papillary configurations.

Von Hanseman's case⁶⁷ lacked the primary testicle tumor, which had been previously removed. Noteworthy are the metastases in the aortic glands; in these cysts, with sero-sanguinolent contents lined with cubical and cylindrical epithelium, pointed to the teratoid nature of the primary growth. (These cysts, according to v. Hanseman, in no way resemble the so-called "lymphatic cysts" sometimes found in the lymph glands.) The metastases elsewhere were typical. For the interpretation of these findings, what has been said in connection with Steinert's case applies.

Askanazy⁶⁸ reported the case sent to Dr. L. Pick by Dr. Salén of Stockholm. Its importance lies chiefly in the systematic study of the various forms assumed by the Langhans cells. Although some portions of the primary tumor are quite typical, transitions, not only to alveolar arrangement of the Langhans cells, but also to duct-like and complex papillary forms, clothing cyst walls, are described. Askanazy concludes that besides the usual typical and atypical types of Marchand the Langhans cells may assume alveolar sarcomatous, carcinomatous, cystomatous, angiomatous or papilliferous forms. Isolated occurrence of this type of cell in teratomata of the ovary and testicle, simulating simple tumors (epithelioma chorionectodermale in the sense of Pick) can thus be accounted for by an excessive growth

of these elements. The chorionepitheliomatous tissue, according to this author, can be identified by its numerous characteristics even when it assumes fantastic or complicated shapes, and moreover, can be directly and unbrokenly traced to simpler forms which correspond to the types laid down by Marchand, and among other constituents contain syncytium, showing a foamy appearance, ciliated margin and a tendency to fibrous transformation.

Scott and Longcope⁶⁸ are the only American authors who reported a case of chorionepithelioma in the male. The testicle was undescended, and contained no teratomatous tissue (only cells of the Langhans type); the metastases were typical.

The last case to be mentioned is that of H. Dillman.⁶⁹ The testicle contained teratomatous, "adenocarcinomatous" and chorionepitheliomatous structures. The latter were represented only in one very minute area.

A case described by Langhans⁷⁰ appears so questionable that I have not included it in the literature. A description of this case, and the metastases of a testicle tumor examined by Eden,⁷¹ which almost positively was a chorionepithelioma, will be found in Risel.⁸⁰ Eden's case is too incomplete to be of real value. Historically it is of interest because it influenced the English commission to form a judgment against the fetal origin of chorionepithelioma in the female.

THE AUTHOR'S THREE CASES.

To these nineteen cases collected from the literature I am able to add three additional cases. The first two are from material kindly placed at my disposal by Prof. F. C. Wood, from the collection of St. Luke's Hospital. The first case was operatively removed seven years ago. At the time, the tumor was classified as a sarcoma of the testis. The second was very recently removed, and given to me by Professor Wood with the diagnosis of chorionepithelioma of the testis. The third case is derived from a tumor of the mediastinum in a patient from the Roosevelt Hospital.

In all these cases the material had been put up in formalin or very weak alcohol, and in no instance was it possible to demonstrate glycogen. Sections were cut by the celloidin method. Besides the routine hematoxylin-eosin staining, picro-acidfuchsin and iron hematoxylin were employed.

CASE 1.—C. P. W., St. Luke's Hospital, operated on Nov. 23, 1897.

History.—The patient was a man 40 years of age. For two years enlargement of one testicle had been noted, the increase being gradual and uniform. Local applications and tapping, at which only a little bloody fluid was withdrawn, proved of no service, but injection of iodine was followed by a marked reduction in size. During the past few months the tumor has again, and more rapidly, increased, and the patient has lost twenty pounds in weight. The tumor was removed. The subsequent history is unknown.

Macroscopic Examination.—The testicle and epididymis are completely fused, forming a mass irregularly quadrilateral in shape, 10 cm. by 10 cm., and 6 to 8 cm. thick. The surface is smooth, with dilated blood vessels showing through the tunica and occasional slight nodular projections. At the upper pole a thick but otherwise normal spermatic cord is found. Its veins are varicose. The color of the entire tumor is a faded greenish-blue from the preservative, in which it has lain for seven years. Sections across the entire growth show several large districts separated by dense, coarse, fibrous septa which converge upward and toward the beginnings of the cord. The largest and most central area has an irregular pitted surface and brownish-gray uniform color. Nearer the cord the tissues are denser, more fibrous, and smooth on the cut surface. At the lower pole the color is more yellowish and mottled yellowish-brown. In almost direct continuity with this portion of the mass is a region of diffuse hemorrhagic appearance which has all the characteristics of the often described "recent thrombotic areas" (Hansemann and others). Small punctate hemorrhages are seen everywhere in this neighborhood. Nowhere are there any indications of cystic structures, bone or cartilage. No testicle substance could be found. Numerous pieces were removed from all parts of the tumor, which was too large for making serial sections.

Microscopic Examination.—The findings are very varied, but certain types of cells in different groupings can be isolated on analysis. The albuginea is normal except for occasional areas of fibrous thickening. From this tunic septa extend into the tumor; the larger strands were already visible macroscopically. By frequent subdivision the strands become thinner and thinner until narrow fibrous bands surround the alveolar portions of the tumor epithelium. No Leydig's cells are seen in the testicle proper; at the beginning of the spermatic cord, which is normal, many of these cells can be observed. The vas is normal. No seminiferous tubules were found.

The macroscopically necrotic areas are formed by a structureless faintly granular mass traversed by bands of fibrin, and occasional areas of degenerating round cells. At the margins of these large areas poorly staining cells of various

kinds occur, some well advanced in a degenerative process, others still showing traces of nuclei and cell outline. There are no large or diffuse hemorrhagic areas, but small extra vascular collections of red blood cells are not uncommon. Except in the old fibrous septa no blood vessels of any size appear. Between the tumor alveoli, soon to be described, occasional capillary vessels may be seen, some of which show a more intimate connection with the tumor cells.



Fig. 1.—Case 1. Tumor of the testicle. To the left is an enormous vacuolated sheet of syncytium. To the right and above is an alveolar collection of Langhans cells surrounded partly by syncytium, which is also found in the center of the alveolus, partly by a fibrous septum. Lower down the syncytium contains isolated Langhans cells, giant nuclei and vacuoles, within which are a few red blood cells.

1. The epithelial cells are polyhedral, with cytoplasm in spots taking a fairly intense eosin stain, especially in the most actively growing portions, in other parts very light or finely granular and transparent. The cell outline is remark-

ably sharp and shows the effect of contact pressure. The nuclei are round or oval, have a sharp nuclear membrane, deeply staining chromatin and one or two nucleoli. The size of the cells varies from 10 to 14 microns, that of the nuclei from 8 to 11 microns. The line of demarcation between the fibrous

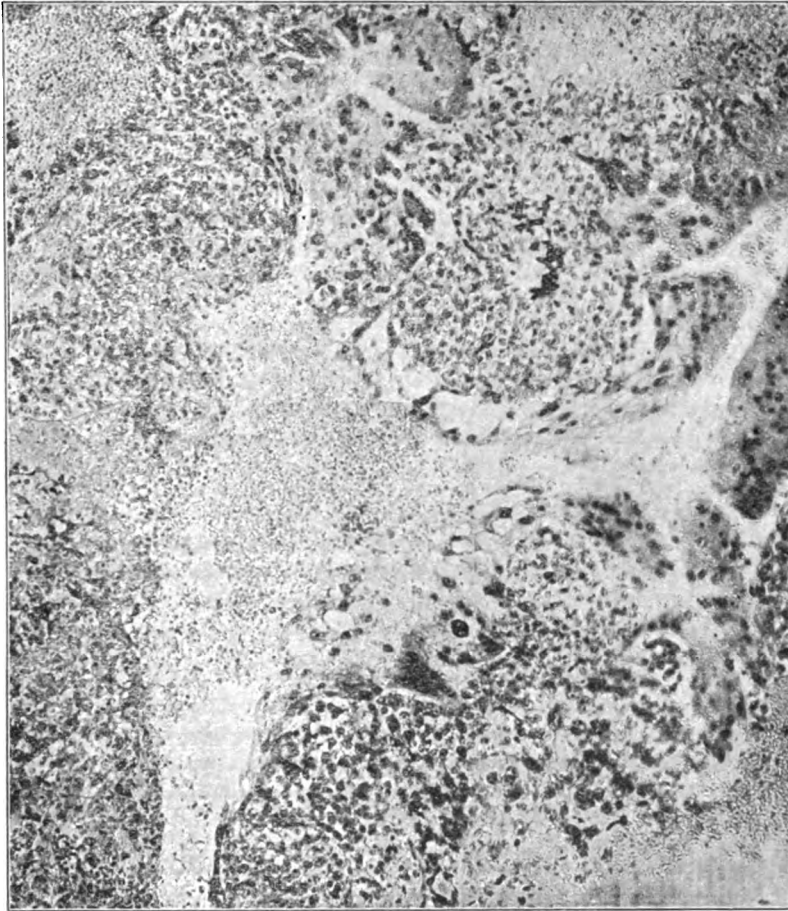


Fig. 2.—Case 1. Tumor of the testicle. Alveolar arrangement of Langhans cells, with intimate relation to large syncytial masses. The syncytium is typically vacuolated and bounds large blood spaces. Transition types abound. At the periphery (left upper corner) are fibrous septa and granular detritus.

stroma and the tumor cells is very sharp. At the edges of the tumor the advancing cells appear in longer narrow columns,

and here and there a few may be seen in the narrow tissue spaces.

2. In some of the alveoli are scattered larger and more deeply staining cells of varied shape, with multiple nuclei. They resemble foreign body giant cells. In other districts enormous protoplasmic masses, reaching 500 to 700 microns in length, and 50 to 100 microns in width, and with very

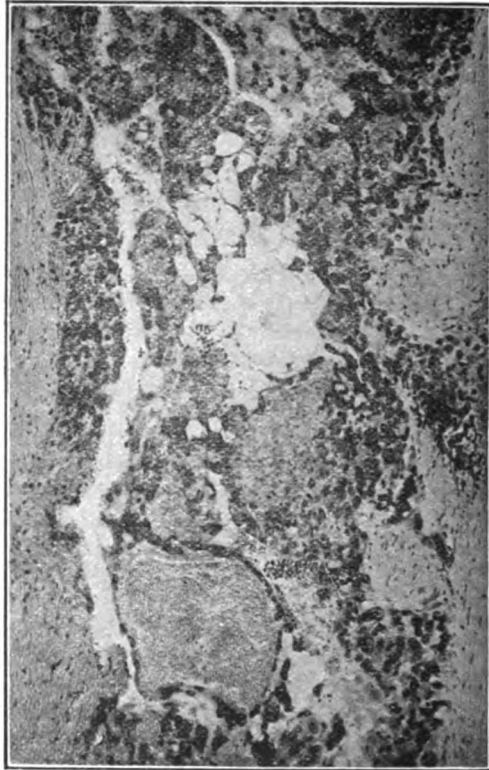


Fig. 3.—Case 1. Tumor of the testicle. Syncytium and Langhans cells as in the typical chorionepithelioma of Marchand; at the edges young connective tissue.

numerous nuclei, are found at the edges of the alveoli, also sending processes between the cells of type 1. These syncytial forms may have a homogeneous, deeply staining protoplasm, but more often a vacuolated and "foamy" appearance predominates. The nuclei vary in size, but on the whole are smaller than those of the main tumor cells, more deeply

staining, and with a closer nuclear network. Usually no nucleoli can be distinguished; no mitoses were seen. Fragmentation, pyknosis, vacuolation, and other degenerative nuclear changes are not uncommon. Occasionally giant nuclei of the most bizarre shapes can be found. Where the foamy or vacuolated cytoplasm is present the nuclei show semilunar or other compression or distortion shapes. Within the syncytium or at its edges are cells of type 1, some well preserved, others degenerating, still others showing a nearer approach to the syncytial type by deeper staining of the cytoplasm and indefinite cell outline (Fig. 1).

3. Sometimes arising from the spreading syncytium, at others independent of it, are narrow, elongated cells reaching a length of 20 to 30 microns with most intensely staining elongated nuclei. These cells may be seen within the alveoli, and also advance into the surrounding fibrous tissue. Whenever they are found the cell growth is very active.

4. Transitional cells of very varied size and appearance are seen arranged like the cells in epithelioma of the skin. The alveoli usually show a rounded outline, and almost invariably contain large and numerous syncytial masses. The cells may be but little larger than cells of type 1; others reach three or four times their dimensions and resemble large and irregular cartilage cells. Their protoplasm is uniformly light, their cell outline sharp. Many are found within the syncytium, their outlines being well preserved. The nuclei are light, show much chromatin, nucleoli and a well-marked nuclear membrane. The most diverse sizes and shapes are encountered. Mitoses are rare, but not entirely wanting (Figs. 2 and 3).

The different quantitative arrangement of these constituents gives an extremely varied picture. The syncytial masses usually have large collections of red blood cells in close proximity; some of the blood cells are also found in the vacuoles of the syncytium, which often acts as a boundary to large blood lacunæ. Capillaries are also seen surrounded by cells of type 1, and may end in the blood lakes just referred to. Their endothelium may be preserved, or only partially destroyed, syncytium or cells of the other types forming the boundary.

No glycogen could be demonstrated in the specimen, which had been preserved for seven years in formalin, later in weak alcohol, but the cells of type 1 morphologically were similar to cells containing glycogen.

To sum up, the tumor was composed of an alveolar epithelial growth which showed a tendency to hemorrhages and necrosis. The tumor elements consisted of cells closely resembling Langhans' cells, syncytium, chorionic wandering cells and transitional forms, arranged in some spots as in the typical form of Marchand,

but yet in the main the alveolar arrangement predominating. Single fields can be found in which no one could distinguish the picture from that of a chorion-epithelioma of the female. This effect is heightened, where, by chance, a projection of the stroma forms a villous-like figure covered with Langhans' cells and syncytium.

No other ectodermic, no mesodermic or entodermic structures were found.

CASE 2.—R. C., St. Luke's Hospital, admitted March 10, 1905, aged 16 years.

History.—The family and previous history of the patient is negative. Two months ago he noticed that the left testicle was larger than the right one, and it has gradually increased until now it has attained the size of an orange. Until one week ago there was neither pain nor discomfort, but since then the tumor is tender on palpation. For the last two nights a dull testicular pain has prevented sleep. There was no loss of flesh and strength, and no other symptoms.

On examination nothing but the swelling of the testicle was found.

The testicle was removed; the wound healed promptly. The subsequent course will be followed, if possible.

Macroscopic Examination.—The testis and epididymis form a single mass about the size of a goose egg. The tunica is smooth and normal; the cord shows no macroscopic changes. On sections the diffuse hemorrhagic appearance is striking. The hemorrhages have not the "thrombotic" form usually noted, but a more arterial, brighter red, and less even carnified consistence. Large areas show a low papilliform surface such as is seen in recent adherent pericardium. Macroscopically no cysts are noticeable. Here and there the hemorrhages are more compact and typical.

The growth had already been cut into several pieces when it reached me. Numerous blocks were prepared and examined.

Microscopic Examination.—Large areas of fairly normal testicular tissue were found. Some seminiferous tubules appear to be functioning; others, nearer the tumor, are undergoing pressure atrophy, and still others are almost unrecognizable and necrotic where embedded in a granular homogeneous mass. In some spots the active tumor tissue is in fairly close proximity to the tubules, but always separated by a considerable layer of fibrous tissue. Nowhere do the seminiferous tubules show an increased proliferative activity or break through into the surrounding stroma. The stroma and Leydig's cells are considerably and uniformly increased.

The tumor proper is separated from the remaining testicular substance by no distinct capsule, but yet there is every-

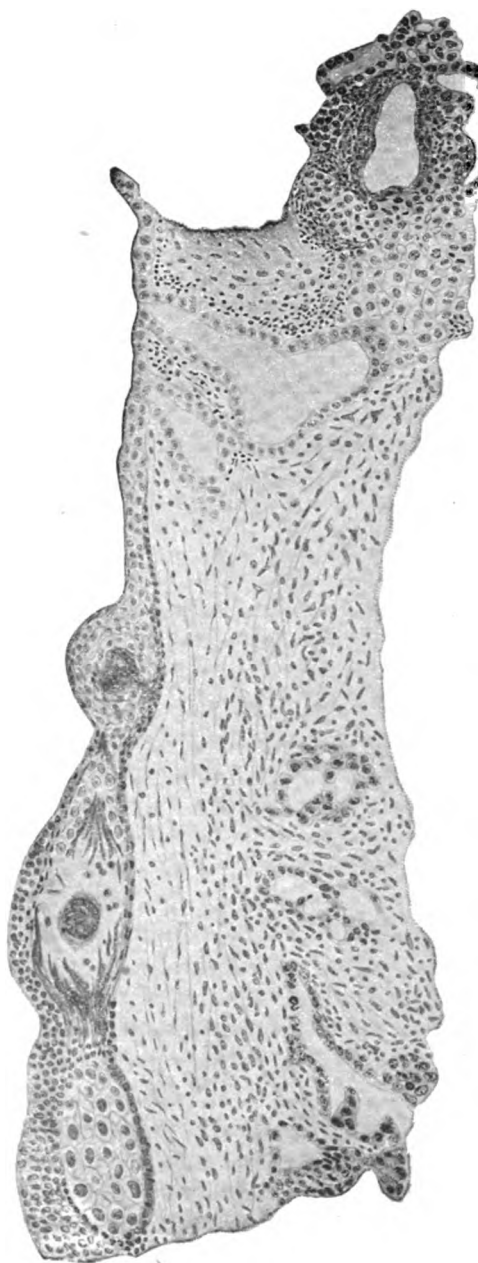


Fig. 4.—Case 2. Teratoma of the testicle. Part of a narrow cavity 8 mm. long is shown. To the left is epidermis with hornified epithelium, merging toward the right into a lower layer of epithelium, which dips down to form two glandlike structures. At the bottom of the deeper gland the epithelium is more darkly staining and fused. In direct continuity is a more alveolar arrangement of the cells, which by unbroken transitions merge into the papilliferous structures, composing the main part of the tumor. To the left and below is one of these papilliferous cavities.

where at least some intervening space filled by fibrous or myxomatous tissue.

The teratomatous nature of the growth is unmistakable. The ectoderm is most typically represented by rudimentary skin in one spot. Here both the stratified stratum corneum and the deeper rete Malpighii are plainly shown lining one

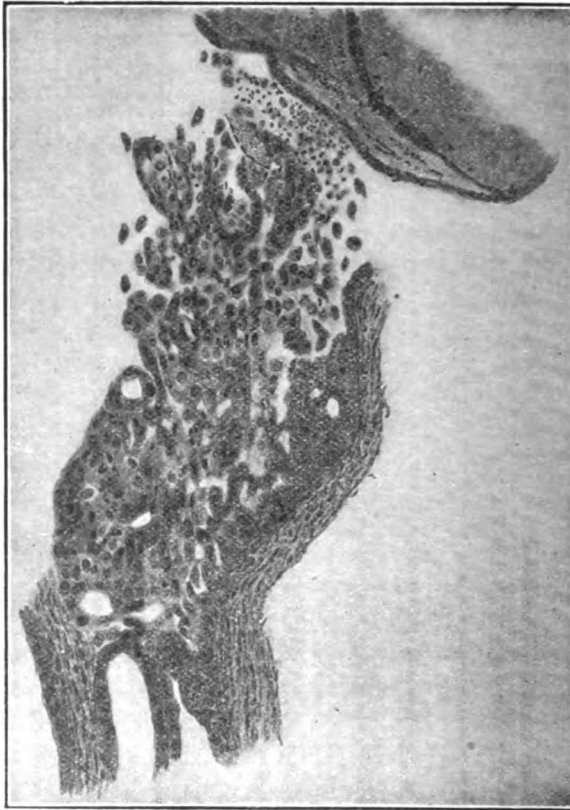


Fig. 5.—Case 2. Teratoma of the testicle. Below, this shows closely aggregated complexes of Langhans cells on a fibrous stroma, (infiltrated with round cells). Above the cells assume the type of chorionic wandering cells and approach a small arteriole (the red blood cells within this are drawn too small) whose wall they invade. At the extreme upper angle is necrotic material and fibrin.

side of an oblong cavity about 8 mm. long, and shading off into a lower multiple layer of epithelium, which finally becomes cubical and single, dipping down to form two simple

glandlike structures and eventually breaking up and diffusely invading the stroma. By means of serial sections a direct connection between the epidermis and the typical papilliferous structures now to be described could be traced, the transition being gradual but unbroken (Fig. 4).

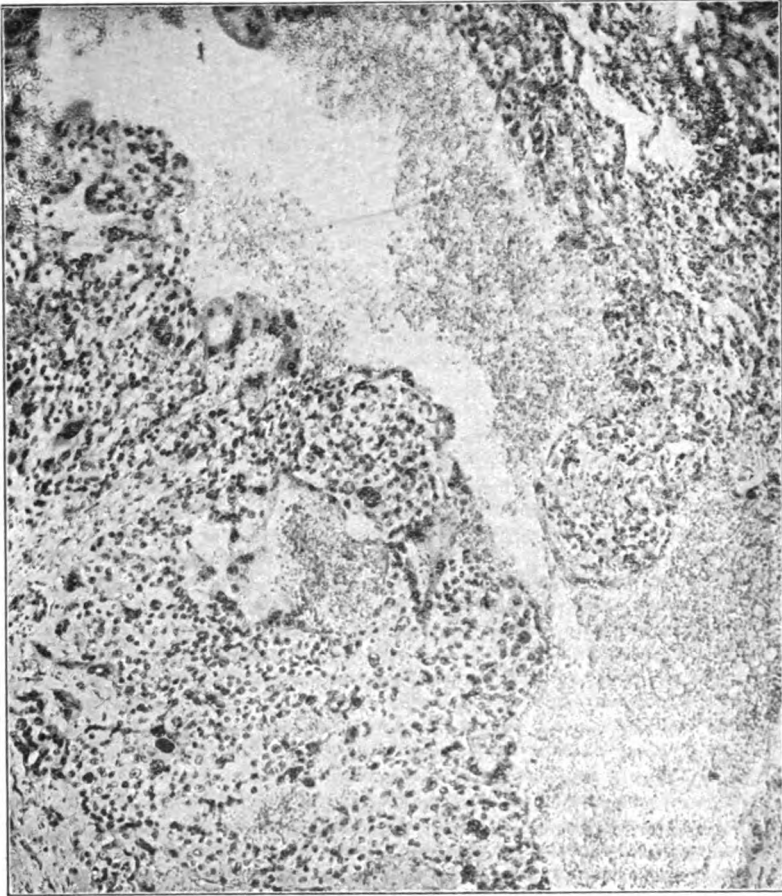


Fig. 6.—Case 3. Liver metastasis. Typical choronepithelioma. In the center is an enormous blood lake bounded by Langhans cells and syncytium. The liver cells are compressed and contain bile-pigment.

Numerous simple cysts, as well as papilliferous cysts, are lined with epithelium. These epithelial cells are cubical or of irregular polyhedral shape, with sharp angular cell outline and clear cytoplasm. The nuclei are large and oval, have a

distinct cell membrane, often one or two nucleoli, and granular chromatin network. Mitoses are common. The size of the cells is fairly uniform, varying between 10 and 14 microns, the nuclei reaching 8 to 12 microns. In many spots the structures are very complex, cysts, true papillæ (shown to be such by serial sections), and rugæ form a bewildering appearance almost similar to that seen in malignant adenoma. On the papillæ the epithelium is often multiple. In numerous places the epithelium invades the stroma, forming cell columns or quite as often assuming a diffuse reticulated distribution. In the neighborhood of hemorrhages or of small blood vessels, the epithelium leaves the main papillæ in an irregular fan-shaped form, the cytoplasm is darker, the nucleus larger, less regular, and the type changes to that of the chorionic wandering cells (Fig. 5). These cells have a nucleus, 10 to 15 microns or more in size, and the cell body may reach as high as 30 microns. These cells abound among the hemorrhagic detritus, and about, or even under, the endothelium of the small vessels, which show incomplete or eroded walls. Among this type of cell numerous smaller and lighter cells similar to those previously described are scattered. No large syncytial masses were found in spite of repeated and thorough search. The epithelium, however, in many regions has a more syncytial appearance due to loss of cell boundaries. About some blood vessels of larger than capillary size, but with very thin walls, the epithelial cells of both types are grouped, so as to give the appearance of a perithelial sarcoma. The cells are closely placed about the vessel, but away from it gradually become further separated into alveolar forms by the connective tissue.

Circular or elongated tubes lined with a high, cylindrical epithelium with small basal nucleus or an occasional goblet cell are found. They have a distinct *membrana propria*, and one or two circular layers of unstratified muscle surround them. These structures were interpreted as entodermal formations.

The mesoderm is represented by fibrous, fibrosarcomatous and true myxomatous tissue, also by scattered unstriped muscle, and by several islets of cartilage.

Of the well preserved portions of the growth by far the greater part is composed of large irregular alveoli, very similar to the alveoli of carcinoma. The stroma has already been described. In some spots it sends fine fibrils into the alveolus, but on the whole any indication of intercellular substance is wanting.

This case must be interpreted as a teratoma containing all three layers, in which chorionectodermal tissues (in the sense of L. Pick) have undergone active proliferation. The Langhans cells line cysts, cover papillæ, surround blood vessels, and assume more diffuse though

never typical alveolar arrangement. Chorionic wandering cells are common; large syncytial formations are not represented. In one spot an unbroken connection between an epidermis-lined cyst and diffusely growing chorionectodermal tissue could be traced.

CASE 3.—N. S., male, aged 21, was admitted to Roosevelt Hospital Sept. 17, 1904; died Oct. 21, 1904.

History.—According to the patient's own account the present illness began three weeks previously with a chill followed by fever. For the last two weeks he has had cough and blood streaked expectoration, with sharp thoracic pain between the shoulder blades, most marked while lying down. There has also been weakness, dyspnea and loss of weight.

On admission the veins of the neck and shoulder of the left side were more dilated than on the right. The heart was displaced slightly downward and to the left. Above the heart was an area of dullness, and a loud systolic murmur could be heard posteriorly at the level of the sixth dorsal vertebra. Anteriorly over the left upper lobe was dullness, bronchial voice, diminished breathing and sibilant and sonorous rales. Similar signs were heard over the right lower lobe posteriorly. The abdomen was distended without the signs of free fluid. The temperature was irregular, hectic, and rose to 104 degrees. On September 29 the patient coughed up half an ounce of pure blood. He died with symptoms of general weakness and dyspnea. On admission the case was diagnosed as one of aortic aneurism; later the diagnosis was changed to that of mediastinal tumor.

An autopsy was performed, but no record kept. Small portions of the lung, liver and necrotic parts of the mediastinal mass were preserved. At no time was any note made of the size of the testicles; presumably they were of normal size.

Macroscopic Examination.—The material from this case consisted of numerous small pieces from the mediastinal mass and nodules from the lung and liver.

The mediastinal growth was of a dark green-brown mottled color, with circular hemorrhagic and necrotic areas of very friable consistency and smooth marble-like surface. The pieces from the lung showed fairly normal lung tissue, which was spongy and contained air. Within these pulmonary tissues irregularly spherical nodules from pea to cherry size were scattered. In color they corresponded to the hemorrhagic areas seen in the mediastinal growth, but they were of firmer consistence and less necrotic.

The portions of the liver preserved were of uniform brownish color, and grossly showed no changes. Glisson's capsule appeared thickened. The nodules were similar in size, color and shape to those found in the lung, except in one spot where a larger and more diffuse area was found. This was less

sharply demarcated from the surrounding liver tissue and showed advanced areas of necrosis centrally. In close proximity to this region were two nodules, one of pea size, the other nearly as large as a cherry, of totally different nature. Irregularly spherical but traversed by fibrous bands, these tumors were white in color and softer in consistency, showing on section a tendency to project above the surrounding liver. Here there were neither hemorrhages nor necrosis.

Microscopic Examination.—With the exception of the two small liver nodules just mentioned the eight nodules of liver and lung were exactly similar. Their outline was fairly sharp. Centrally, in each, was a large, hemorrhagic area composed of degenerating red blood cells, fibrin, detritus and almost unrecognizable cell material. Peripherally was a cellular area of varying width formed by clear polyhedral epithelial cells strongly similar to those of the Langhans type, with large and typical syncytial masses. The syncytium showed numerous well preserved nuclei, vacuolation and protoplasmic processes. Moderate numbers of transitional types were present. Large and small blood lacunæ abounded near, or surrounded by, the syncytial masses. The arrangement was that of the typical chorionepithelioma of Marchand. The surrounding lung tissue in some portions was normal; in others the alveoli were filled with a pneumonic exudate. In some regions a fibrous septum of considerable thickness, probably antedating the tumor, separated healthy and diseased area. The liver tissues show much bile pigment in all the liver cells. Where the tumor and liver tissue about the difference in cell types is very distinct. The liver trabeculæ are dark, filled with pigment granules and have a homogeneous appearance. The Langhans cells stand out clear and lighter and show neither pigment granules nor dark coloring. The syncytium also is distinct; its nuclei larger and darker than that of the liver cells; its cytoplasm vacuolated and more granular. Although in many places in direct contact, no continuity with liver tissue could be found. The liver cells often show the effect of pressure. There are marks of chronic congestion and Glisson's capsule is thickened. A few outlying vessels are filled with Langhans cells (Fig. 6).

The two whitish metastases are composed of a single type of cell somewhat smaller than those of the zellschicht, with clear though more deeply staining cytoplasm and similar nucleus. They are arranged like the cells of an alveolar sarcoma, the fibrous tissue entering and subdividing in the very large irregular alveoli. At the periphery the cells in small groups invade the liver substance, crowding aside the liver cells. The contrast between the pigmented liver cells and the clear, light tumor constituents is very striking.

The pieces from the mediastinal tumor show formless necrotic tissue.

As the testicles were not examined, and the parts of the mediastinal tumor submitted to the microscope showed only necrosis, the genesis of this tumor can not be cleared up. Very possibly a more careful autopsy might have demonstrated a mediastinal dermoid or teratoma. The metastases are absolutely characteristic of chorionepithelioma.

3. HYDATIDIFORM PROLIFERATIONS IN TERATOMATA.

CASES IN THE MALE.

The preceding cases, many of them consisting of testicle tumors in which chorionepitheliomatous proliferation with teratomatous elements are demonstrable, are sufficiently numerous, similar and well authenticated to be accepted as a distinct and recognized variety of tumor. Schlagenhauser has, in addition, described what he considers a modification of these neoplasms, which bear a similar relation to chorionepithelioma of the testicle that hydatid moles bear to chorionepithelioma of the uterus. This author has personally reviewed the case reported by Karl Breus⁷² in 1878, and re-examined the material, also collecting five cases from the literature. All these cases have certain features in common. A primary testicle tumor is present in all; in most of them the testicle tumor is evidently a teratoma; in one only (Breus' case) do parts of the metastases resemble chorionepitheliomatous tissue. In all, metastatic extension into the large veins has taken place, usually reaching as far as the heart. The intravascular growths show, macroscopically at least, a striking resemblance to hydatid mole. If this resemblance is to be accepted as more than a merely superficial similarity, the microscopic findings must coincide with the picture seen in hydatidiform degeneration of the chorion, particularly in regard to the appearance and behavior of the ectodermic covering. In order to discuss this question, which has certain important theoretic features, a review of these cases is necessary, but as both Schlagenhauser and Risel give a full review and no new cases have been added, a condensed account will suffice.

Although not the first in point of time, it is well to consider Breus⁷² case, as reviewed by Schlagenhauser, before the others.

The autopsy showed a large testicle tumor in a man of 40. The tumor in addition to sarcomatous and carcinomatous por-

tions contained cysts of diverse structure. The cord had attained the size of a thumb. Its dilated varicose veins were filled with narrow strands which could be traced into the internal spermatic vein and from it into the inferior cava, where the strand was 1.5 cm. in diameter, and had numerous club-shaped appendages.

In the lungs many large and small metastases were noted, the new growths being composed chiefly of syncytial forms with occasional scattered Langhans cells. According to Schlagenhauser the nodules should be regarded as villous emboli. Small branches of the pulmonary artery contained thrombi similar to those to be described below. In the left auricle, starting from the fossa ovalis, was a slimy, grapelike vegetation comparable to an hydatid growth. The small transparent masses had a more opaque center. In the right heart a similar picture was noted, but here the mass was directly continuous with a strand of the thickness of a finger, projecting into the inferior cava and forming a skein which filled the entire chamber and projected through the open foramen and into the left heart. The intravascular growths assumed a complicated glove-like form composed of myxomatous and fibrous tissue covered by an epithelium which resembled both the Langhans and syncytial type. Within the grape were cysts clothed with cubical and cylindrical epithelium.

His conclusions are:

1. The primary tumor is a teratoma.
2. The grape-like vegetations are in part equal histologically to an hydatid mole.
3. The lung metastases are the result of villous emboli, and where the epithelial covering has proliferated, a chorionepitheliomatous tissue has been formed.

Summing up, he considers this case and the four others yet to be discussed as teratomata in which an hydatid-like proliferation within the vessel has taken place because the fetal membranes or their rudiments have undergone an intravascular hydatidiform degeneration. To account for this change occurring not in the primary but only in the intravascular portions of the growth, Schlagenhauser assumes that the ectodermal elements are here in a physiologically favorable condition, as, in a normal pregnancy, the fetal ectoderm physiologically has the property of invading the blood vessels. In the blood spaces the syncytial masses predominate, for it is their chief function to supply nourishment to the rest of the cells by osmotic processes.

Waldeyer⁷⁸ reported his case as a myxo-chondro-sarcoma. In the spermatic cord hyaline, worm-like structures, attached by

pedicles to the vessel walls, occupied the veins. The covering epithelium of these "hydatids" was continuous with that of the vessel walls and in spots was "heaped up" as if to form new pedicles.

Silberstein's¹⁴ case showed intravascular structures similar to those in the case of Schlagenhauser. The myxomatous groundwork of the grapelike formations contained cysts lined with epithelium. The testicle tumor probably was a teratoma.

The testicle tumor described by Kanthack and Pigg¹⁵ was evidently teratomatous. The enormous glandular metastases contained many cysts. In the great venous trunks and heart the hydatid-like growths were composed of fairly firm fibrous tissue and enclosed epithelial lined cysts.

MacCallum,¹⁶ according to Risel¹⁰ (p. 135), no longer regards the tumor he reported a lymph-endothelioma of the testis. The primary tumor may possibly belong among teratomata with very unequal development of the fetal layers. The widely distributed intravascular growths (re-examined by Risel) show a myxomatous groundwork, a cellular covering, continuous with that of the vessels, and cystic structures within the grape. Nowhere is chorionepitheliomatous tissue in evidence.

DISCUSSION BASED ON THE PRECEDING CASES.

Of all the cases, Breus-Schlagenhauser's should be most seriously considered. More important than the main intravascular growths are the small villous thrombi from which he directly traces chorionepitheliomatous nodules. If these findings are accepted, they would correspond exactly to the pictures seen in the metastases of a malignant hydatid mole. Risel¹⁰ (page 132) objects to a genetic or even morphologic identity with these structures, claiming—undoubtedly with justice—that simple myxomata (two cases of Marchand) can assume hydatid-like forms within the large blood vessels through purely mechanical causes; but this fact would not necessarily exclude the possible occurrence of true hydatids under similar conditions. A stronger argument against the claims of Schlagenhauser is the presence of minute cysts, and not sharply circumscribed cell complexes, within the grape-like masses, and also the fibrous, more often than myxomatous, stroma, which lends weight to the assumption that these intravascular metastases have no greater specific significance than the teratomatous metastases in Steinert's or Hansemann's cases. Schlagenhauser represents the epithelium covering the villi, in his case, as of both the type of Lang-

hans' and syncytial cells. The mention of a "heaping up" of the covering epithelium (Waldeyer) might be regarded as the plasmodial buds seen on normal villi. I am unwilling to assume as positive a stand against Schlagenhauser as that taken by Risel, especially as the case of Pick, immediately to be discussed, must also be considered; but, on the whole, the burden of proof still rests against the assumption that hydatid growth occurs in teratomata.

If we accept Schlagenhauser's view, the theoretical deductions justified in regard to the genesis are not far-reaching. To assume that the presence of degenerated fetal membranes would point to a derivation from an impregnated pole cell instead of from a blastomere is not warranted, as was pointed out by Pick, Steinert, etc. If subsequent observations confirm the findings of true hydatidlike growths it will merely afford another proof of the specific nature of the ectodermal growth, which shows so many characteristics, both physiologic and morphologic, of the chorionepithelium of pregnancy.

HYDATID DEGENERATION IN A TERATOMA OF THE FEMALE.

The most interesting and also most convincing case bearing on this subject and corresponding to a non-malignant hydatid in a teratoma, is the one described by Pick,⁷⁷ but in this instance also a reasonable amount of doubt remains, and further confirmation will be required to set the question at rest.

On opening the abdomen of a woman of 30, who was operated on for ectopic gestation, in addition to the tube, which contained a few chorionic villi, a dermoid was discovered. The dermoid was typical of its kind; but within a subsidiary cyst of some size and completely separated from the rest of the growth as well as from the ectopic sac, were numerous hydatid bodies, which in both their gross and microscopic morphology corresponded to intrauterine hydatidiform degeneration of the chorion.

In discussing this case, Pick believes that the origin of the hydatid from the tubal pregnancy can be absolutely excluded. The growth is separated everywhere by a fibrous cyst wall; the formation is old; the tubal pregnancy recent. He regards it as a part of the original dermoid "anlage," a closed portion of the membrana chorii, the cyst as a portion of the intravillous

space filled with serous fluid instead of blood. This case, in Pick's opinion, confirms Schlagenhauser's thesis, except that the question of a pole cell vs. blastomeric origin can not be decided by this or similar observations. What it does show is that Marchand's view, that a congenital anlage arises at the earliest formative stage, is correct.

With Risel, I hold that this case is open to some doubt. If the early tubal pregnancy had not produced the hydatid formations, this by no means excludes an origin from a previous pregnancy, or unnoticed abortion, by a blood channel later obliterated. It may be objected that a limitation of such metastases to one spot, and, at that, to such an inaccessible one as the center of a dermoid cyst, is inconceivable. I will acknowledge that it is unlikely, impossible it is not. Further evidence is necessary finally to settle this question.

4. CHORIONEPITHELIOMATOUS PROLIFERATION IN TERATOMATA ELSEWHERE THAN IN THE GENERATIVE GLANDS.

The chorioneplitheliomatous proliferations so far dealt with have all, with the exception of Boestrom's case and perhaps my third case, had their origin from the generative glands. Boestrom has not, as far as I know, conclusively shown that the testes were normal, and, as no teratoma of the brain was found, the case is therefore incomplete and can not be classified with any certainty. Bearing the genesis of these tumors in mind, we might expect to find a chorioneplithelioma wherever teratomata occur, as first suggested by Schlagenhauser, and a few observations on record fulfill this expectation.

The case mentioned by Albrecht,⁸ in which the epithelium lining the branching ducts in a teratoma of the liver closely resembled chorioneplithelium, is reported with too little detail to permit the forming of a serious judgment. Judged, however, by what this author says, his assumptions are not justified.

The most convincing and unique case of chorioneplitheliomatous proliferation outside of the generative organs (except that of Djewitzki, which will be next considered and in which no teratoma was found), is Ritchie's.⁹ This case was also examined by Teacher,¹⁰ who vouches for the chorioneplitheliomatous nature of the growth.

In a man 24 years of age, who died of a malignant tumor of the anterior mediastinum, a large neoplastic mass was found jutting from the root of the left lung. The mass was partly cystic and partly solid. The large cyst contained the usual dermoid constituents—hair, sebaceous glands, epidermoid structures, muscle, etc.—and was in direct continuity with the solid part of the growth. The solid portions proved typical chorionepithelioma. The numerous small nodules scattered throughout the lung were of identical composition.

The description is that of a true chorionepithelioma from a teratoma. The illustrations of this paper bear out this contention. As good a judge as Teacher agrees with the findings. The teratoid nature of the growth is apparent even if entoderm was not found. This case appears conclusive and incontrovertible evidence in favor of the occurrence of chorionepithelioma elsewhere than in the generative glands.

Djewitzki⁸⁰ reports a case of chorionepithelioma of the bladder, which also appears convincing, but has not quite the value of the previous case.

A virgin, 75 years of age (menopause twenty years previous), was curetted for hemorrhage, the curetings showing hypertrophic endometritis due to fibromyomata. Death from cardiac failure permitted an autopsy. At this was found metastases in the lungs, bronchial glands, spleen and sigmoid, arising from a small nodular tumor situated immediately beneath the mucous membrane of the bladder. All the tumors were typical chorionepitheliomata; no teratoma was found. The uterus contained only the fibroids previously diagnosed.

Djewitzki scouts the idea of a teratoma with overgrowth of the other constituents by the chorionectoderm. As a possible genesis, he suggests carrying along some of the cloacal ectodermic cells by the Wolffian duct. Another alternative he offers is the reversion to an embryonal type, or *entdifferenzierung* of the bladder epithelium.

The extreme age of the patient makes any connection with a possible concealed or denied pregnancy unlikely. A latent period of twenty or more years has previously been unknown. That the author has overlooked teratomatous constituents in his examination of the tumor is possible, or that such tissues were obliterated is also conceivable. I am inclined to regard this case, with the slight reservation already mentioned, as another instance of chorionepithelioma in the relation of consanguinity (in the sense of L. Pick) to its host. The extremely long latent period of nearly seventy-five years is in

strong contrast with the usual course noted in tumors, which are classified as congenital.

**5. A DETAILED DISCUSSION OF THE MORPHOLOGY AND
OF THE THEORETICAL BASIS OF CHORION-
EPITHELIOMATOUS TUMORS.**

HISTOGENESIS.

The foregoing has shown that a certain number of tumors of the testicle, also a tumor of the mediastinum, and one of the bladder, which bear a close resemblance to the chorionepithelioma of the female, are on record. Since Schlagenhauser published his views on the significance of these growths they have been classified as chorionepitheliomata. Where in direct connection with a teratoma and where the typical characteristics of the growth are well developed, but little difficulty will arise in recognizing their nature. Their recognition will become correspondingly difficult when teratomatous constituents can not be demonstrated (as in my first case), and where, through unknown causes, deviations from the so-called typical type of Marchand appear. In such cases it would be desirable to have one or more distinctive or pathognomonic characteristics in mind to decide the question; and we now must try to determine whether such exist.

As has just been stated, the embryonal nature of the tissue can often be shown by its occurrence in a teratoma. Its ectodermal origin has been conclusively proven in a number of cases—in Pick's ovarian teratoma in a girl of 9 years, in Risel's case, in both of which the syncytium was in direct continuity with neuroepithelium, and in my own Case 2, in which the cells of Langhans type were continuous with the epidermis, and then diffusely invaded the stroma, gradually, but without a break, merging into the papillary and atypical type of chorionepithelioma. This continuity proves merely that we are dealing with an embryonal ectodermic tissue, but does not show that the cells are specifically chorionepithelium. To look for such highly specialized characteristics as the hair found in the epidermis, or the nerve cells found among the brain substance of embryos, would be futile, for the chorionepithelium is a primitive tissue, and normally never reaches a higher stage of development.

SYNCYTIUM, ITS VALUE AS A DISTINGUISHING MARK.

At one time it was supposed that the syncytium would prove a sufficiently specific structure to assure the diagnosis, but subsequent observations have inclined some authors to doubt this. In the first place, the term syncytium has been very loosely employed. According to Bonnet,⁸¹ multinuclear protoplasmic masses should be divided into plasmodia, masses which have not yet undergone subdivision; syncytia, composed of a fusion of pre-existing separate cells; and symplasma, or cells which are undergoing a degenerative process during which, at a certain stage, a real or apparent fusion exists. In pathologic material we are unable to decide whether we are dealing with the first or the second, and as far as chorionepithelioma is concerned, this is of no significance if we accept Marchand's view that syncytium and Langhans' cells are merely an expression of various functional activities of the villous covering; we even do not know which is the first to appear in the human embryo. Symplasma should be recognized by the degenerative changes, most readily appreciated in the nuclei, where they take the form of fragmentation, pyknosis, etc.

The most typical syncytium has a deeply staining homogeneous protoplasm, with well-preserved and numerous nuclei, vacuoles often containing red blood cells, and, under favorable conditions, a ciliated margin (Pick, Wlassow, etc.). Syncytial formations have been found in various inflammatory conditions, such as pneumonia and nephritis (Aschoff⁸²), in carcinomata and endothelioma (Recklinghausen⁸³), in adenocarcinomata gigantocellulare of the liver (Babes⁸⁴), in the uterine mucosa in ectopic gestation (Schmidt⁸⁵), etc., which seems to rob them of much of their specific or diagnostic value. Another possible source of error has been pointed out by Sternberg,⁸⁶ who showed that material preserved in formal-Müller lost some of the sharp cell outline, and consequently could simulate syncytium; but this artefact would still be unable to account for typical arrangement, etc. A careful study of thin sections stained by various methods will often show a tissue to be composed of discrete cells, which in thick sections or to cursory examination will appear fused and syncytial. Whether the syncytia or giant cells described by these various authors really ever so exactly correspond to or simulate

the syncytium of true chorionepithelioma, with its cytologically distinctive marks (vacuolization, ciliary margin, etc.), I hesitate to affirm.

I have a tumor of the arm from a man of 77, which is a polymorphous-celled carcinoma composed of large, often polynuclear cells, which resemble syncytium, but yet do not answer to all the characteristics which should be considered necessary. Such a tumor should not be classed as a chorionepithelioma, but, on the other hand, should also not be used as a possible proof against the specific nature of syncytial formations.

DOUBTFUL TUMORS.

The tumor of the liver described by Marx⁸⁶ gives the impression of being a chorionepithelioma; whether primary or secondary must be left undecided; that of Michel certainly must be considered of chorionepitheliomatous nature (or chorionectodermal, to employ the nomenclature of Pick, who subjects it to a convincing critique⁸⁷). Neither of these articles offers any evidence against the doctrine of chorionepithelium in teratomata.

Sternberg⁸⁸ recently reported a tumor of the testicle in a man of 26, which caused metastases in the liver, lungs and retroperitoneal glands. Both tumor and metastases were composed of light cubical cells about the capillary blood vessels, and of syncytial masses in close connection with the vascular endothelium. He classifies this tumor as a sarcoma in the sense of Malassez and Monod with vasoformative function, without supplying any new facts, except that he claims to have found syncytium arising from the maternal endothelium in young human embryos. Sternberg concludes that many, if not all, so-called chorionepitheliomata in teratomata are sarcoma or endothelioma. In the discussion which followed no additional facts were brought out. It will be of interest to see whether the promised fuller report will prove more convincing.⁸⁹

CHARACTERISTICS OF THE NORMAL TROPHOBLAST.

But for the sake of excluding all sources of error, let us set aside syncytium as a pathognomonic structure. We then fall back on certain general or group characteristics to distinguish the chorionepithelium. It is true that the further the tumor tissue departs from its normal prototype the more difficult and questionable will its recognition become. It is by a careful study of the normal covering of the villi and of the chorionepitheliomata of the female, in direct connection with pregnancy, that we can alone hope to recognize the similar tumors, in-

dependent of gestation, arising from a teratomatous growth.

The normal trophoblast shows three varieties of cell forms—the Langhans type or *zellschicht*, the so-called chorionic wandering cells, and large plasmodial or syncytial bodies. Even in the chorion of pregnancy these constituents vary in their numerical relations and arrangements, not only during different stages of pregnancy, but also in individual cases. These variations become more marked as we turn to the pathologic formations, such as hydatids and chorionepitheliomata. The normal trophoblast shows certain physiologic properties (morphologically recognizable), which distinguish it from other normal tissue (unless we except the single instance of the erosive action of the osteoclast during normal bone processes), in that it invades the structures of the uterus and erodes them. This invasion, though physiologic, corresponds exactly to the invasion of malignant tumors, although usually, unlike these growths, it reaches a definite end. But in the malignant neoplasms of pregnancy—hydatid and chorionepithelioma—the process goes further, boundless and destructive proliferation results, with ultimate harm to the whole organism. This erosive action of the normal chorionectoderm follows along certain well-defined lines. The cell columns invade the decidual and also muscular tissues after the type of a carcinoma, or more diffusely so as to simulate sarcoma, sending the active chorionic wandering cells far into the structures of the host. These cells appear attracted by the blood vessels, erode their coats, creep along beneath the vascular endothelium, finally entering the lumen and causing perivascular hemorrhages. As the rapid increase in number of cells is not accompanied by a corresponding, in fact, by any, formation of new vessels, the tissues would at once be subject to lack of nutrition and consequent necrosis, were not some osmotic or other transfer of nutritional substances possible. This osmotic function would seem to be attended to by the large syncytial sheets or surfaces which, according to Bonnet, not only where in direct contact with blood, but also when more distantly removed, show a hemoglobin contents, in distinction to the other trophoblastic cells, which show such blood pigment only at their edges, where postmortem diffusion might account for it. Hofbauer⁹⁰ has actually shown the loosely com-

bined iron in the basal layer of the syncytium, and has followed it into the stroma of the villus.

In the pathologic forms already referred to, necroses form a large part of the tumors, as the vascular supply, especially in the metastases, is often insufficient; wherever, however, the blood supply is abundant and readily reached, as in the liver and lungs, the metastases show well-developed and prominent syncytial forms. In addition to the characteristics so far brought forward, the chorionectoderm appears to exercise a destructive (lytic, if I may call it so) action on neighboring cells independent of pressure necrosis, and accompanied by the extensive formation of fibrin, which last may very possibly be traced to the hemorrhages.

To recapitulate, the normal trophoblast is composed of three cell forms, which proliferate rapidly, invade the blood vessels, cause hemorrhages, necrosis and fibrin formation, and having fulfilled their function during gestation show no further malignant properties. These same cells appear in the malignant growths of pregnancy, evince the same properties, but fail to stop before causing harm to the host. Once having entered the blood vessels, rapid and fatal metastases occur, metastases often faithfully copying the primary growth. Such tumors, whether they assume the typical or atypical form of Marchand, are recognized and accepted when in direct connection with pregnancy, partly because of this relationship, partly because they so faithfully copy the normal trophoblast. Were we to analyze each cell, nothing more characteristic would be arrived at than the analysis of a single cell taken, let us say, from a carcinoma or from a sarcoma. Again, no one property here mentioned is strictly limited to this form of growth. Sarcomata are often hemorrhagic, carcinomata metastasize through the blood vessels (Goldmann,⁹¹ etc.).

Such properties as the glycogen contents of the Langhans cells are shared by many varieties of growths, although Lubarsch⁹² is inclined to consider it a sign, though not a sure one, of the embryonal derivation of a tumor. Attempts to use the size, by actual measurement, of a cell or its nucleus, as a means of comparison or identification, are, of course, of uncertain value.

"TYPICAL" AND "ATYPICAL" FORMS OF MARCHAND.

The description given by Marchand of the two main types of tumor found in connection with pregnancy is

so excellent and applies so well to chorionepithelioma in teratoma, that I will quote it in full.

"Those cases are typical in which the characteristics of the chorionepithelium which appear in the first period of gestation are represented, with no or but slight variation. They show well developed, continuous syncytial masses of the form of irregular, multinuclear strands and branching protoplasmic processes, and with more or less well developed and numerous transparent polyhedral cells of the structure of the zellschicht. Whether the Langhans cells can be wholly absent, as has been described in some cases, I am by no means certain. If they are not found in some spots, that is no proof that they are completely wanting."

"Those cases are atypical in which everywhere, or at least almost everywhere, the chorionepithelium has lost its peculiar normal grouping, and occurs only as isolated cells, which may show various forms. The cell masses which form the surface and inner layers of the decidua serotina (basalis) in hydatid mole may be taken as an example or pattern of these forms. The cells rarely show the habitus of the delicate, transparent membranous and sharply circumscribed zellschicht elements, with regular, oval nuclei, frequent mitoses and glycogenic cell protoplasm. More often their habitus is that of compact, more deeply staining and very irregularly shaped cells, with nuclei varying greatly in size, sometimes attaining enormous proportions and showing intense and often equal staining properties, which allow the recognition of their syncytial character. These elements may form multinuclear aggregates, but in many cases no large syncytial masses develop. Transitions between the two chief varieties may occur if, here and there, larger syncytia are formed."

VARIATIONS FROM MARCHAND'S TYPES ENCOUNTERED IN TERATOMATA.

Further on in the same article, but without emphasizing this point as markedly, Marchand says: "Tumors of the chorionepithelium may show certain resemblance with ordinary carcinomatous tumors." In the testicle particularly, this large alveolar type may predominate, the chorionepitheliomatous type cropping out at some small spot or first becoming manifest in the metastases. The testicle tumors described by Ribbert³³ (p. 619) impressed Pick as probably chorionepithelioma, and their further study might show typical formations. The growth described by Askanazy³⁶ and my own Case 2 vary still further from the accepted types of chorionepithelioma, as the Langhans cells line cystic and papilliferous configurations hardly even suggestive of the group under discussion, but betraying their true nature by entirely typical spots which are in direct continuity with the

papillary formations. Lately, Pick²³ has reported a case of carcinoma of the liver in a female, which he claims is a true chorionepithelioma because of its morphologic characteristics. His explanation is based on the theory that this apparently simple tumor is a teratoma with its other tissues undeveloped. Although far from denying the possible truth of his conclusions, the proof offered in this or in similar observations, if more should be forthcoming, is very weak unless we accept the group characteristics previously discussed as sufficiently convincing, even when unaccompanied by a demonstrable teratomatous origin. It is also tempting to generalize and use such a case as an argument for the embryonic derivation of all tumors (or the converse, as an emphatic proof against their parasitic origin, as Pick has done).

IS CHORIONEPITHELIOMA A "SPECIFIC" TUMOR?

There is another possible aspect of the discussion to which I turn with great hesitation, as it involves not only the debatable question of metaplasia, but also the "specificity" of chorionepitheliomatous tissue. We have seen that syncytium and Langhans cells, or at least structures morphologically their equivalent, in direct continuity with unequivocally ectodermal tissues, have been noted by Pick, Risel and myself. Pick and Risel both regard this fact as a proof that the anlage from distinct fetal membranes, such as Schlagenhauser has assumed, is not a requisite. Why not generalize still further, as Lubarsch²⁴ has done, and consider it merely as an evidence of a metaplastic change of any embryonal ectodermic structure, which under certain unrecognized conditions is capable of producing this variety of tumor tissue, just as in response to other stimuli it undergoes carcinomatous changes or degeneration (Tautfer,²⁵ Yamagiva²⁶). By this view we would regard the chorionepithelioma as a secondary malignant degeneration of a teratoma, or, where no teratoma is found, a degeneration of the tumor "*Keim*," which gives rise to chorionepithelioma or carcinoma, depending on the nature of the stimulus. Against this view only one valid argument can be advanced, but it is of almost overwhelming force, and that is the coincident metastasizing of other teratomatous constituents. To explain the composite metastases various hypotheses have been offered (see above), the most plausible being the carrying into the circulation of tumor cells which are closely related to, or the equivalent of, blastomeres, and therefore able to

differentiate into all three embryonic layers. As in the resulting composite metastasis, the chorionepithelium is again the only actively "malignant tissue," we must assume that the stimulus causing this excessive proliferation is general and evenly distributed within the organism so as to act on the newly formed metastasis at this new site, which is unlikely; or that the blastomeric metastasising cell contains among its other potentialities that of forming chorionepithelium, which therefore again attains the dignity of a specific tissue.

It would seem, therefore, that this way of regarding the question, and it is not as an advocate of either metaplasia or anaplasia that it has been suggested by me, but merely as a possible train of reasoning, offers no further light on the subject and, in fact, leads us back directly to the other hypothesis. It does, therefore, strengthen, in a negative way, the conception of chorionepithelium as a specific type of tissue, as an integral part of the teratoma, and not merely as an accidental conglomeration of cells, which bear a superficial resemblance to the trophoblast through some vagary of growth. Of still greater and, in fact, of decisive value would be a convincing case of hydatid proliferation in a chorion: epithelioma; for if the chorionepitheliomatous tissue is shown to undergo the same degeneration as the tissues of the true chorion in pregnancy, no one could deny its exact and specific equivalency. As yet the case of Breus-Schlagenhauser is the only one which approaches this requirement, and as long as these hydatid-like forms are still under suspicion of being a merely accidental imitation due to physical forces, it can not be used in evidence.

6. CLINICAL ASPECT AND SYMPTOMS OF CHORION-EPITHELIOMA OF THE TESTICLE.

Of the 22 above-cited cases, including my own, the 4 cases of Wlassow are without any clinical data. Schlagenhauser's has but very scanty observations; Schmorl's Case 2 at the time it was reported was too recent to be of service, likewise my own Case 2. In Steinhaus' case and my Case 1, the subsequent course could not be ascertained; consequently, only 13 cases can be utilized in trying to present the course of this variety of tumor. Boestrom's case and my Case 3, respectively, presented the symptoms of a tumor of the brain and tumor of the mediastinum, without a primary neoplasm of the testis being convincingly excluded.

The youngest patient was 16 years of age, the oldest 43; but the great majority were found in young adults in the early twenties. As the local testicle symptoms were absent or at most consisted of enlargement of one testis with very slight pain, the patients more often presented themselves on account of abdominal symptoms due to the metastases, and when all hope of a radical cure had passed.

The average duration of the disease can not be accurately determined, for the patients were unable to state when the testicular enlargement had begun, or in some cases this enlargement was never sufficient to attract attention. The longest duration was that of my Case 1, in which the testicle had been appreciably increased in size for two years. Probably the usual course will be found to be less than twelve months. Trauma was repeatedly followed by a sudden increase in size and the appearance of the metastases.

The symptoms varied greatly, but, except for such local discomfort due to the testicle tumor, which in a few cases reached the dimensions of a child's head, were confined chiefly to the abdomen and lungs. The retroperitoneal glands along the iliac fossa, and those situated higher up along the aorta, caused intestinal and stomach pressure symptoms. In one case only was jaundice noted; in several others the liver palpated, greatly enlarged and nodular. A transient edema of the leg followed operation in the case described by Carnot and Marie. Cough, dyspnea, blood-streaked or bloody expectoration were almost constant terminal symptoms, readily accounted for by the frequency of the lung metastases. The high fever in my Case 3 was probably due to necrotic mediastinal and lung tumor masses; in Scott and Longscope's patient the symptoms on admission were those of a tubercular pleurisy. The involvement of the kidneys was never manifested clinically. Risel's Case 2 had a transverse lesion of the cord due to the pressure of the metastatic tumor tissue. Toward the end, loss of weight and general cachexia were prominent symptoms in all.

Clinically, chorionepitheliomata of the testicle, therefore, show no very distinctive course. Like other malignant tumors of these organs, they are rapidly fatal; metastases, both in near and in distant organs, occur early and usually are too far advanced when first seen by the surgeon to permit of a radical cure.

7. SUMMARY OF OBSERVATIONS AND DEDUCTIONS BASED ON THEM.

1. In the female, direct continuity of malignant and non-malignant hydatids and of true chorionepitheliomata with the epithelial covering of the fetal villus has been repeatedly observed, thus proving these tumors to be composed of fetal ectoderm.

2. A similar continuity of tissues has been noted in the covering of deported villi, which acted as the starting point of metastases, arising from hydatids or chorionepitheliomata. This affords additional proof that the metastases are likewise derived from the fetal ectoderm.

3. Theoretical considerations point to the similarity of the embryologic processes which produce the normal fetus and the embryoma or teratoma. The theory of Marchand and Bonnet traces the origin of these growths from impregnated pole cells or early blastomeres, which are liberated from the cell complex, remain latent for a longer or shorter period, and then, in response to as yet unknown stimuli, resume their growth with the still inherent property of forming all the structures found in the normal embryo.

4. The resulting tumors, genetically the twin of their host, may be highly complex, imitating the normal embryo in a startling fashion; or very simple, so that they afford but little clue to their teratomatous origin (single tooth, thyroid tissue, etc.). Transitions between the complex (embryoms, teratomata, dermoids) and simpler forms (bideroms, mixed tumors, etc.) can be traced (Bonnet). Such teratomatous growths have been found in the ovary, testis, retroperitoneal space, mediastinum, brain, sacral region, etc.

5. Among the tissues found in these tumors, ectodermal structures often take a leading part. Neuroepithelium, skin and chorionepithelium have been found in direct continuity surrounded by the most diverse tissues. Wherever teratomata occur chorionepithelium may be looked for (though, of course, it does not occur in the majority of cases).

6. Consequently, these ectodermal structures are of equal significance, and it becomes unnecessary to assume a separate anlage of fetal membranes to account for the presence of chorionectoderm.

7. To the chorionectoderm, in teratomata, one must also accord a distinct and specific significance, like that accorded to a special tissue such as skin or brain sub-

stance, because in most of the recorded cases it shows all of the characteristics of the trophoblast of pregnancy. These characteristics are not only morphologic (the three types of cell forms—Langhans cells, syncytium, chorionic wandering cells—absence of vessels, etc.), but also physiologic (vaso-destruction, fibrin formation, glycogen production).

8. As in the chorionepitheliomatous tumors in direct connection with pregnancy, the chorionectoderm in teratoid tumors also assumes atypical forms, which render its recognition difficult, and perhaps, in some cases, impossible.

9. These atypical forms may present a tissue similar to the so-called atypical form of Marchand, or, as the result of an excessive growth of Langhans cells, a more diffuse alveolar carcinomatous, papillary, cystic, or perithelial arrangement may be found.

10. The true nature of these atypical forms of tumors can be recognized by discovering teratomatous constituents and typically chorionepitheliomatous portions, in direct continuity with, or in gradual but unbroken transitions to, the less characteristic parts.

The foregoing chain of evidence is not weakened by the arguments advanced by certain authors. Granted that syncytium is not confined to chorionepithelium alone (although typically foamy syncytium with vaso-destructive tendency and ciliary margin has not been found elsewhere), the recognition of chorionepithelium is not based on any single morphologic fact! True to its prototype in normal gestation, the chorionepithelium evinces a high proliferative activity, shown not only in the primary tumor, in the simple metastases, but appearing also in the composite teratomatous metastases, in which it again forms the "malignant" element.

To expect the chorionepithelium to differentiate into higher tissues, like the other tissues dating from "the earliest anlage" of the teratoma, is as reasonable as to look for a similar differentiation of the placental tissues, which are also examples of cells derived from "the earliest anlage," and which nevertheless retain their early embryonal characteristics throughout the long period of gestation.

In order to interpret the close connection of the chorionepithelial tissues as a "vasoformative" function and to derive their genesis from the endothelium of the blood vessels (as was done by Malassez and now again by

Sternberg), it is necessary to ignore their direct continuity with certain undoubtedly ectodermic structures, such as epidermis or neuroepithelium.

I wish to thank Prof. T. M. Prudden for placing the resources of his laboratory at my disposal, and also for much interest and advice during the progress of this study. I am greatly indebted to Prof. F. C. Wood for the material of my two testicle cases, and for much aid and many helpful suggestions. Dr. Ludwig Pick (of Berlin) has very kindly given me valuable advice and criticism through several private communications, for which I take this occasion to thank him.

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89. Since the completion of this article the promised article by Dr. C. Sternberg has appeared in the Zeitschr. f. Heilkunde, April, 1905, vol. xxvi, No. 4, p. 105 (Ein peritheliales Sarkom (Haemangioendotheliom) des Hodens mit "Chorionepitheliomartigen Bildungen"). As it represents a formulated attack on the doctrine of specific chorionepitheliomatous tissue in teratomata, foreshadowed in the discussion of his case presented at the pathologic congress in 1904, it deserves to be mentioned.

The original testicle tumor was removed from a man of 26 years, who died two months later. Metastases were found in the lungs, liver, retroperitoneal glands, besides tumor masses floating in the lumen of the inferior vena cava. The primary growth was composed of various sized nodules made up of narrow cell columns, either arranged singly or at most in double rows about fine blood capillaries, and by the juxtaposition of these columns imitating cystic or glandlike structures. Protoplasmic masses, such as are usually described as typically syncytial, are in evidence, but not numerous, among the tumor cells and also within the blood vessels, at times completely filling the lumen. No continuity with testicle tissue could be found. The metastases in liver, lungs and glands show a more marked development of syncytial forms, which preserve the same intimate relation to the blood vessels, and a less prominent participation of the smaller elements than in the primary focus.

By reason of serial sections, Sternberg concludes that "the de-

scribed tumor elements, the protoplasmic masses, are abnormal anlagen of blood capillaries." This leads us back to the opinions of Malasses and Monod. Sternberg then rapidly glances over the case literature and chooses the cases of the French authors, Rissel's Case 2, and Boestrom's cases as examples of such in which no teratomatous elements were found. He then takes as his premises an arbitrary proposition: "It therefore remains to be shown," he says, "whether the demonstration of the repeatedly mentioned, large protoplasmic, multinuclear and vacuolated cellular bands and clumps justifies the diagnosis of a teratoma, or whether their derivation from the fetal ectoderm is to be regarded as proved." Sternberg next states that tumors of most varied histogenetic origin (carcinomata, endotheliomata) show syncytial formations, and that to consider such structures alone, without other characteristics of the chorionepithelium (absence of blood vessels, hemorrhages, etc.) chorionepitheliomata is unjustified. Further, that in the normal placenta some of the syncytial elements very probably are of maternal origin. Finally, that the chorionepithelium represents a structure of the "first ectodermal anlage," which in teratomata is unrepresented, the ectodermal tissues consisting of differentiated structures. The coincidence of teratomata with these endothelial sarcomata is accidental, and occurs most frequently in the testis and ovary, because here teratomata are more common.

In reply I would say that no one has attempted to make the diagnosis of teratoma by means of syncytium alone. Where no teratomatous elements were found the tumor tissue showed the very characteristics demanded by Marchand. In the less typical cases, such as Askanazy's and my own Case 2, other teratomatous elements were demonstrated. One of the strongest advocates of the specific nature of chorionepithelioma in teratomata (L. Pick, Virch. Arch., vol. clxxx, No. 1, p. 176), acknowledges this occurrence of syncytium in other tumors, also the possibility of maternal syncytium in placentation. The objection advanced by Sternberg, that chorionepithelium is a tissue of "the first ectodermal anlage," which otherwise does not occur in teratomata, falls to the ground when we consider that this tissue, though of "the first anlage," retains its undifferentiated structure throughout the entire course of pregnancy (i. e., that its first anlage is also its permanent condition), and that complex teratomatous metastases can be explained only by assuming the presence of still undifferentiated cells within the primary teratoma (call them Keimgewebe, blastomeres, as you will), which also represent tissue of this "first anlage."

One point, and this has been a crucial one, has been entirely neglected by Sternberg. Even if he could find justification in interpreting the syncytial formation as endothelial in origin, how does he account for their occurrence in direct continuity with other undoubtedly ectodermal tissues, such as neuroepithelium?

Sternberg has therefore discovered no new facts which undermine or even weaken the position taken by Pick and others, who claim that chorionepithelioma in teratomata has a "specific" value. He has merely again interpreted the genesis of syncytial forms from the vessel endothelium, as did Malasses and other French authors, without offering new proof in support of this old contention.

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